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THE ACUTE INHALATION TOXICITY OF PYROLYSIS PRODUCTS OF HALON 1301

FINAL REPORT

BRUCE E. LEHNERT

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FOREWORD

This final report summarizes investigations conducted in the project entitled "Acute Inhalation Toxicity of Pyrolysis Products of Halon 1301". During the course of the studies, investigations were undertaken to address numerous objectives. Specific information provided in this report, accordingly, is formatted in the context of the objective(s) to which it pertains. Each Section is prefaced by a statement of the objective(s) it concerns, a brief summary of accomplishments and findings that are detailed in the section, and a citation of reports and publications that have emanated in total or in part from research performed during the accomplishment of the objective(s). Each Section begins with its own Introduction, which is then followed by a Methods and Materials section, a Results section, and a Summary or Discussion section.

A summary of the project's overall objectives and the Sections in which they are addressed in this Final Report are as follows:

SECTION A

Objective 1: To develop exposure systems and methodology to deliver pure, stable atmospheres of HF, HBr, or HCl to laboratory rats.

Objective 2: To determine the toxicological equivalency of inhaled HF, HBr, and HCl in the upper and lower respiratory tract.

Objective 3: Characterize the anatomical sites of injury caused by HF, HCl, and HBr when inhaled at high mass concentrations through the nasal (NB) and oral (MB) pathways.

Objective 4: To assess the relative toxicities of the above halides when inhaled at high mass concentrations during mouth and nose breathing.

SECTION B

Objective 5: To characterize the upper and lower respiratory tract lesion(s) produced by HCl inhalation in the nose breathing (NB) and mouth breathing (MB) rats during CO_2-induced increased minute ventilation.

SECTION C

Objective 6: To examine the toxicity of HF when administered directly into the rat's lung.

SECTION D

Objective 7: To determine if work performance incapacitation occurs after acute high concentration inhalation of HCl via the nose (NB) or the mouth (MB).

Objective 8: To assess post-exposure exercise as a potentiator of the severity of expression of halide-induced respiratory tract injury.

SECTION E

Objective 9: To initiate studies to determine whether or not lung injury becomes more pronounced after halides are breathed in combination with a particulate phase.

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Lehnert, B.E.: Physiological and biochemical endpoints in inhalation toxicology. International Programme on Chemical Safety, World Health Organization, Hannover, FRG, October 6-October 9, 1992.

Oberdörster, G., Ferin, J., Finkelstein, J., Baggs, R., Stavert, D.M., Lehnert, B.E.: Potential health hazards from thermal degradation events: Particulate vs. gas phase effects. SAE Technical Paper Series, The Engineering Society for Advancing Mobility Land Sea Air and Space, pp. 1-15, 1992.

Oberdörster, G., Ferin, J., , Baggs, R., Stavert, D.M., Lehnert, B.E.: Health hazards from thermal degradation events: Particulate vs. gas phase effects. Intern. Conference on Environmental Systems. Aerospace Medical Association, Seattle, WA, July, 1992.

Brainard, J.R., Kinkead, S.A., Wood, G.O., Stavert, D.M., Lehnert, B.E.: Potential involvement of hydrofluoric acid in perfluoroisobutylene-induced lung injury. 1992 Annual Meeting of the Society of Toxicology, Seattle, WA, February 23-27, 1992.

Stavert, D.M., Archuleta, D.C., Behr, M.J., Lehnert, B.E.: Relative acute toxicities of hydrogen fluoride, hydrogen chloride, and hydrogen bromide in nose and pseudo-mouth breathing rats. Fund. Appl. Toxicol. 16:636-655, 1991.

Archuleta, D., Martinez, M., Stavert, D.M., Lehnert, B.E.: Pathologic responses to inhaled HCl during periods of increased minute ventilation in pseudo-mouth breathing and nose breathing rats. 1991 Society of Toxicology Annual Meeting, Dallas TX, February 25-March 1, 1991. The Toxicologist 11(1): A339, 1991.

Lehnert, B.E., Kinkead, S.A., Kress, J.D., Kober, E.M., G.O. Wood, Stavert, D.M., Brainard, J.R.: Mechanism(s) of Lung Injury Caused by Perfluoroisobutylene (PFIB) and Related Agents. In: Proceedings of the 1991 Medical Defense Biosciences Review. pp. 273-291, 1991.

Brainard, J.R., Kinkead, S.A., Kober, E.M., Stavert, D.M., Lehnert, B.E.: Potential involvement of HF in mechanisms of pulmonary toxicity of perfluoroisobutylene. In: Proceedings of the 1990 Scientific Conference on Chemical Defense Research , 1991.

Archuleta, D., Stavert, D.M., Lehnert, B.E.: Pathologic responses to HF, HBr, or HCl inhaled by pseudo-mouth breathing and nose breathing rats. 29th Annual Meeting of

the Society of Toxicology, Miami Beach, FL., February 12-16, 1990. The Toxicologist 10:A818, 1990.

Kusewitt, D.F., Stavert, D.M., Ripple, G., Mundie, T., Lehnert, B.E.: Relative acute toxicities in the respiratory tract of inhaled hydrogen fluoride, hydrogen bromide, and hydrogen chloride. 28th Annual Meeting of the Society of Toxicology. Atlanta, Ga, February 27-March 3, 1989. The Toxicologist 9:A144, 1989.

Lehnert, B.E., Stavert, D.M.: Pulmonary pathophysiology of oxides of nitrogen, and pulmonary pathophysiology of halide gases. Military Medical Pulmonary Research Review and Analysis, Ft. Fitzsimmons, Denver, CO, September 20-22, 1988.

SECTION A

Objective 1: To develop exposure systems and methodology to deliver pure, stable atmospheres of HF, HBr, or HCl to laboratory rats.

Results: A polyethylene chamber and delivery system was fabricated to accommodate up to 12 animals held in non-avoidance and non-constraining animal holding tubes. Also, exposure and measurement techniques were developed for these atmospheres.

Reports:

Stavert, D.M., Archuleta, D.C., Behr, M.J., Lehnert, B.E.: Relative acute toxicities of hydrogen fluoride, hydrogen chloride, and hydrogen bromide in nose and pseudo-mouth breathing rats. Fund. Appl. Toxicol. 16:636-655, 1991.

Objective 2: To determine the toxicological equivalency of inhaled HF, HBr, and HCl in the upper and lower respiratory tract.

Results: Respiratory tract injury due to the inhalation of high concentrations of the halides HF, HCl, and HBr is confined to the nasal cavity of the obligatory nose breathing rat. The severity of injury in the nasal cavity in response to HF, HCl, and HBr generally scale with exposure concentration with the most pronounced level of injury occurring after the 1300 ppm exposures. Histopathologic evidence and body weight changes observed after exposure to the halides suggest that HF is more toxic than are HCl and Br, whereas the responses to HCl and HBr were similar.

Reports:

Stavert, D.M., Lehnert, B.E.: Cross-ventilating rat model: Pulmonary functional status of participating animals. J. Aerosol Med: Deposition, Clearance, and Effects in the Lung. 1:51-66, 1988.

Kusewitt, D.F., Stavert, D.M., Ripple, G., Mundie, T., Lehnert, B.E.: Relative acute toxicities in the respiratory tract of inhaled hydrogen fluoride, hydrogen bromide, and hydrogen chloride. 28th Annual Meeting of the Society of Toxicology. Atlanta, Ga, February 27-March 3, 1989. The Toxicologist 9:A144, 1989.

Lehnert, B.E., Stavert, D.M.: Pulmonary pathophysiology of oxides of nitrogen, and pulmonary pathophysiology of halide gases. Military Medical Pulmonary Research Review and Analysis, Ft. Fitzsimmons, Denver, CO, September 20-22, 1988.

Lehnert, B.E., Stavert, D.M., Archuleta, D.C.,: Cross-ventilation rat model for determination of the deposition, uptake, distribution, and elimination kinetics of inhaled substances. 1986 Society of Toxicology Annual Meeting, New Orleans, LA, The Toxicologist 6:A138, 1986.

Objective 3: Characterize the anatomical sites of injury caused by HF, HCl, and HBr when inhaled at high mass concentrations through the nasal (NB) and oral (MB) pathways.

Results: A unique tracheal tube and mouthpiece was fabricated to allow delivery of HF, HCl, and HBr directly into the lower respiratory tract of unanesthetized rats. The device was shown to have minimal effect on the normal ventilatory patterns of rats. Tissue injury following the nose breathing of these halides was confined to the nasal region. Mouth breathing the halides caused higher mortality, and major tissue damage in the trachea. More peripheral damage was manifested by lung gravimetric increases and histopathologic changes primarily in the larger conducting airways.

Reports:

Stavert, D.M., Archuleta, D., Behr, M.J., Lehnert, B.E.: Relative acute toxicities of hydrogen fluoride, hydrogen chloride and hydrogen bromide in nose- and pseudo-mouth-breathing rats. Fundam. Appl. Toxicol. 16:636-655, 1991.

Archuleta, D., Stavert, D.M., Lehnert, B.E.: Pathologic responses to HF, HBr, or HCl inhaled by pseudo-mouth breathing and nose breathing rats. 29th Annual Meeting of the Society of Toxicology, Miami Beach, FL., February 12-16, 1990. The Toxicologist 10:A818, 1990.

Objective 4: To assess the relative toxicities of the above halides when inhaled at high mass concentrations during mouth and nose breathing.

Results: Development of the "pseudo-mouth" breathing rat model. Demonstration that HF, HCl, and HBr are quantitatively similar in their toxic effects in the respiratory tract.

Reports:

Stavert, D.M., Archuleta, D., Behr, M.J., Lehnert, B.E.: Relative acute toxicities of hydrogen fluoride, hydrogen chloride and hydrogen bromide in nose- and pseudo-mouth-breathing rats. Fundam. Appl. Toxicol. 16:636-655, 1991.

Archuleta, D., Stavert, D.M., Lehnert, B.E.: Pathologic responses to HF, HBr, or HCl inhaled by pseudo-mouth breathing and nose breathing rats. 29th Annual Meeting of the Society of Toxicology, Miami Beach, FL., February 12-16, 1990. The Toxicologist 10:A818, 1990.

Lehnert, B.E.: Physiological and biochemical endpoints in inhalation toxicology, International Programme on Chemical Safety, World Health Organization, Hannover, FRG, October 6-October 9, 1992.

INTRODUCTION

Hydrogen fluoride (HF), hydrogen chloride (HCl), and hydrogen bromide (HBr) gases can be generated as pyrolysis products from numerous halogenated materials, and they can be encountered in a variety of other industrial and nonindustrial settings (e.g., Mayer and Guelich, 1963; Kaleinfeld, 1965; Wohlslagel et al., 1976; Rosenthal et al., 1978; Terrill et al., 1978; Turbini and Zado, 1980; Kaplan et al., 1984). Unfortunately, information about actual mass concentrations to which humans may be exposed to the above halides in various fire scenarios or otherwise has been limited by the fact that existing procedures for their direct analyses are relatively complex and time consuming, and automated instrumentation for determining their concentrations in exposure atmospheres have yet to be developed (Gad, 1990). Regardless, the high water solubility of the halides suggests that they would be both rapidly and efficiently absorbed in the upper respiratory tract during nose breathing in a manner that may protect the more distal lower respiratory tract from their toxic actions, even when inhaled at relatively high mass concentrations. Indeed, in the obligatory nose breathing rat, nasal deposition efficiencies greater than 99.9% have been measured with up to 176 mg/m^3 (215 ppm) HF (Morris, 1982). Although the deposition efficiencies of HCl and HBr in the nose have not been as well characterized, previous preliminary investigations in our laboratory (Kusewitt, 1989), as well as investigations by others (Rosenholtz, 1963; Morris, 1979; Buckley, 1984), have shown that the respiratory tract injury produced by the acute inhalation of relatively high mass concentrations of these latter halide gases is localized mainly to the nasal passages. Hence, it would appear that HF, HCl, and HBr are all efficiently absorbed in the nasal passages during nose breathing, at least in obligatory nose-breathing experimental animals. Although similar studies with any of the above halides have not been performed in the human, confinement of injury to the nasal passages when the halides are breathed via the nasal route would also be expected, given the high absorption efficiency of the human nose (e.g., Pattle, 1961; Speizer and Frank, 1966; Morgan and Frank, 1977; Aharonson et al., 1974).

Unlike with exposures during nose breathing, the injurious responses in the lower respiratory tract to HF, HCl, and HBr when inhaled via the oral route have received essentially no experimental attention, even though a significant fraction of humans are routinely mouth breathers (Niinimaa et at., 1981), and inspired airflow partitioning from the nasal route to the oral pathway in otherwise predominantly nose breathers occurs during a wide variety of human activities (e.g., Camner and Bakke, 1980; Proctor, 1977; Niinimaa et al., 1981; Saibene et al., 1979). Using the rat model, one of the objectives of the present study, accordingly, was to comparatively characterize the anatomical site(s) of injury caused by HF, HCl, and HBr when

acutely inhaled at high mass concentrations through the nasal and oral pathways. Additionally, we have attempted to assess the relative toxicities of the above halides when inhaled at equivalent high mass concentrations during mouth and nose breathing.

METHODS AND MATERIALS

Overview of Experimental Design: Male Fischer-344 rats (SPF, 245-270 g) were lightly anesthetized uniformly with a short-acting anesthetic and either fitted with mouthpieces with attached silastic endotracheal tubes (mouth breathers, MB) or allowed to awaken without further manipulation (nose breathers, NB). After recovery from anesthesia, the NB and MB animals were placed into partial body flow plethysmographs and attached to an exposure chamber. The animals were provided clean, filtered air for 5 min while pre-exposure ventilatory parameters were measured. Exposure atmospheres consisting of either filtered air (controls) or ~ 1300 ppm of HF, HCl, or HBr were then delivered to the rats for 30 min with the ventilatory parameters of the animals being measured over this exposure period. After cessation of the exposures, clean air was again delivered to the animals for 15 min prior to their removal from the plethysmographs. Following the exposures, the mouthpieces and tracheal tubes were removed from the MB animals. MB and NB animals were euthanized 24 hrs after the exposures for histologic analyses of their upper and lower respiratory tracts and for lung gravimetric measurements.

Mouthpiece and Tracheal Tube: The mouthpiece was fabricated from the tip of a polyethylene centrifuge tube. The tracheal tube portion was made from soft silastic tubing, Figure 1. A linear flow resistance of 0.11 cm H_2O/ml/sec was obtained with the device up to 20 ml/sec. The device had a deadspace volume of 0.3 ml. These values closely match the nasal resistance and deadspace values found for 250-g Fischer-344 rats (Stavert and Lehnert, 1988). The devices were intratracheally positioned in rats lightly anesthetized with Ethrane (enflurane, Airco, Madison, WI) using a modified otoscope, and they were secured into place via incisor tooth grip holes drilled into the mouthpieces. When positioned, the endotracheal tube of the device extends ~9 mm past the larynx of a 250-g rat, i.e., approximately mid-tracheally. After the devices were in place, nose clips were attached to the external nares and a rubber band was placed over the mouth of each animal to prevent dislocation of the mouthpiece during exposure. Preliminary studies were performed to determine how the mouthpiece affects the ventilatory patterns of awake rats. These pilot studies included 30-min exposures to filtered air while recording minute ventilation (V_E), tidal volume (V_T), and breathing frequency (f) of both NB and MB rats. Measurements of total pulmonary resistance (R) and dynamic lung compliance

(C_{dyn}) of normal nose breathing animals and of animals fitted with the mouthpieces were made on separate groups of pentobarbital-anesthetized rats while they inhaled filtered air over a 30min penod using methods described elsewhere (Stavert and Lehnert, 1988).

Exposure Atmospheres: HCl and HBr exposure atmospheres were generated by mixing pure HCl or HBr (Matheson Gas, LaPorte, TX) with anhydrous HEPA-filtered air in a stainless steel mixing chamber. The exposure atmospheres were then delivered to the animals using a previously described quartz glass exposure system (Stavert and Lehnert, 1990). HF (4%, Matheson Gas, LaPorte, TX) was mixed with filtered air in a polyethylene mixing chamber and delivered to the animals within a polyethylene exposure system with a design similar to the quartz glass unit. Exposure concentrations of HF, HCl, and HBr were determined by quantitatively drawing samples of the atmospheres through midget impingers (SKC Inc., Eighty Four, PA) at a rate of 400 ml/min. Ionic strength adjusting buffer (ISA) or total ionic strength adjusting buffer (TISAB) was used as collection media in the impingers (depending on the electrode used), and samples were analyzed for a given constituent with calibrated, ion-specific electrodes (Orion Research, Inc. Cambridge, MA). A minimum of three samples was collected and measured for every 30 min exposure. HCl exposure concentrations of 1284 ± 16 ppm and 1293 ± 36 ppm (~ 1499 mg/m^3) were delivered to nose and mouth breathing rats, respectively. HF exposure concentrations of 1295 ± 25 ppm and 1294 ± 67 ppm (~823 mg/m^3), and HBr exposure concentrations of 1300 ± 23 ppm and 1298 ± 21 ppm (~3328 mg/m^3) were delivered during nose and mouth breathing experiments, respectively. Based on previous studies using the animal exposure system, we estimate that the equilibration time (t_{99}) for achieving the required concentrations of the halides in the system was ~35 sec (Stavert and Lehnert. 1990).

Absorption of the halides into the plastic mouthpieces during the exposures of MB rats to HCl, HF, and HBr was determined after every exposure by immersing the mouthpieces into ISA or TISAB for a 24-hr period and then measuring the concentration of a given constituent with the appropriate ion-specific electrode. Total absorption of 0.5, 0.6, and 1.6% of HF, HBr, and HCl exposure atmospheres, respectively, occurred during the mouth breathing experiments. New mouthpieces were used for every exposure.

Ventilatory Measurements: Animals were exposed to the atmospheres while contained in partial body flow plethysmographs, Figure 2, that were attached to the exposure chamber. Prior to the actual exposures, the rats were trained daily for 3 days to sit quietly within the plethysmographs. The plethysmographs consisted of a nose section, head section, body section, and a flow resistance plug. The nose section allows for the attachment of the

plethysmograph to the exposure system manifold and was made of quartz glass for the HCl and HBr exposures and of polyethylene for the HF exposures. The head section, made from Teflon®, contains rubber dam diaphragms which effectively seal the body of a rat into the plethysmograph. The head portion also contains a neck brace to positionally maintain the head of a rat in the diaphragms. The body section of the plethysmograph contains the body of the rat and attaches to the nose section and the flow resistance plug via O-rings. The flow resistance plug consists of a port covered with four layers of 400-mesh SS cloth which provides a resistance to flow created by the expansion and contraction of the thorax within the body section of the plethysmograph. Another port provides attachment to a differential pressure transducer (± 2.5 cm H_2O, Validyne Engineenng Corp., Northridge, CA) connected to an automated data acquisition and analysis system, which consists of an IBM PC running ASYST (Adaptable Laboratory Software, Rochester, NY) software tools and custom software. The system is able to continuously measure ventilatory patterns of up to six rats within separate plethysmographs and convert these signals to breath-by-breath measurements of minute ventilation (V_E), breathing frequency (f), and tidal volume (V_T).

Animal Sacrifices, Lung Gravimetric Measurements, and Tissue Processing: The body weights of the animals were measured immediately before exposure and at the time of euthanization. Rat euthanizations were initiated by ip injections of 50 mg pentobarbital sodium. Following decapitation, the nasal cavity regions were prepared as descnbed by Young (1981), Figure 3. The trachea and lungs were excised, and the heart, extrapulmonary mediastinal tissue, and the esophagus were removed. The lungs were blotted dry and weighed (lung wet weight, LWW). The bronchus leading to the right cranial lobe (RCL) was ligated with fine suture, removed and weighed (right cranial lobe wet weight, RCLWW). [Note: Only the LWW of the experimental animals are presented in this study inasmuch as changes in this parameter were paralleled by RCLWW changes]. Following the gravimetric measurements, the trachea of each tracheal-lung preparation, minus the RCL, was cannulated with an 18-gauge needle that was secured with ligature and the tissue sample was subsequently infused and fixed at a constant pressure of 30 cm H_2O with 10% formalin in phosphate buffered saline for 48 hrs. The RCL were oven-dried to a constant weight at 100°C for 36 hrs and reweighed (right cranial lobe dry weight, RCLDW).

For the histologic analyses of the lung, each left lung lobe was sliced on the same plane as the main-stem bronchi from its apex to its base along a line between the most posterior to the most anterior aspects to expose the maximal planar surface area for sectioning (Stavert et al., 1986; Stavert and Lehnert, 1990). The blocks of tissue were embedded in paraffin and 4-µm sections were prepared and stained with hematoxylin and eosin by conventional methods. The

tracheae of the experimental animals were likewise processed and sectioned longitudinally for histologic analyses of pathologic changes present along their entire lengths.

Semi-quantitative Histopathology of the Nasal Compartment: Injury in the nasal passages was graded according to severity and distribution of a given pathologic feature. Hallmarks of injury included epithelial necrosis of lamina propnal structures, the appearance of proteinaceous and cellular exudates, the infiltration of polymorphonuclear leukocytes (PMN) in the epithelium and lamina propria, and hemorrhage into the lamina propria. A grading scale was used to index the relative extent or distribution of an abnormality within a given tissue section. The distribution index for a pathologic feature ranged from 1 to 4 with 1 = focal. 2 = few foci, 3 = moderate to many foci. and 4 = diffuse distribution. The severity index was used to describe the relative extent of injury, the amount of abnormal material present, and the relative numbers of cells abnormally occurring at affected sites. This index also ranged from 1 to 4 with 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe.

Semi-quantitative Histopathology of the Trachea: Injury along the full length of the trachea was graded according to the severity and distribution of a given pathologic feature in a manner identical to that followed for scoring histopathologic changes in the nasal compartment.

Semiquantitative Histopathology of the Lungs: Histopathologic assessments of the conducting airways focused on analyzing for evidence of epithelial and submucosal necrosis and other signs of an injurious response, e.g., exudates, PMN, and extravasated erythrocytes. These features were scored according to the scoring system used for the nasal and tracheal compartments. Histopathologic assessments of the alveolar region focused on the appearance of fibrin, accumulations of PMN and the relative abundance of alveolar macrophages (AM), the presence of intraalveolar erythrocytes, and alveolar cuboidal cell hyperplasia, i.e., Type II cell hyperplasia (Stavert et al., 1989; Lehnert and Stavert, 1990; Rombout et al., 1986; Warnock, l 982). A grading scale was also used to quantitatively describe the relative magnitudes of each of the above alveolar pathologic features in terms of their: (1) distribution, i.e., relative number of affected pulmonary acini; (2) severity, or the relative number of periterminal bronchiolar alveolar structures affected; and (3) intensity, the relative amount of abnormal material or relative alterations of cells in the alveoli. The distribution index for a given pathologic feature ranged from 0 to 4 with 0 = not observed, 1 = focal in appearance, 2 = few but multifocal, 3 = moderate number to many involved terminal airways, and 4 = all or nearly all acinar structures were affected. i.e., diffuse. The relative severity index for a given pathologic feature ranged from 0 to 4 with 0 = no abnormality, 1 = the focal appearance of the abnormality in

FIG. 1. Mouthpiece used to simulate mouth breathing in the otherwise obligatory nose-breathing-rat. The funnel-shaped component of the device contained holes to accommodate the incisor teeth.

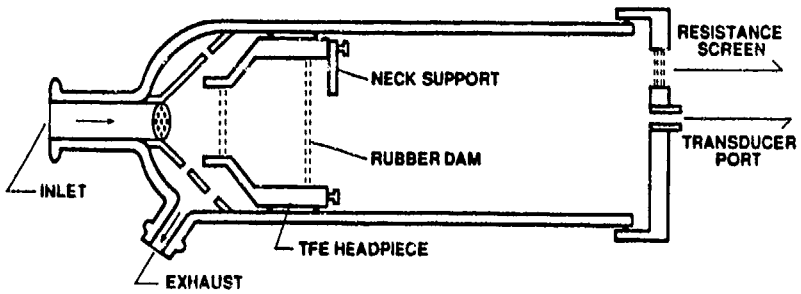

FIG. 2. Partial body flow plethysmograph used for exposing the pseudo-mouth-breathing and nose-breathing rats to air or the halide-containing atmospheres.

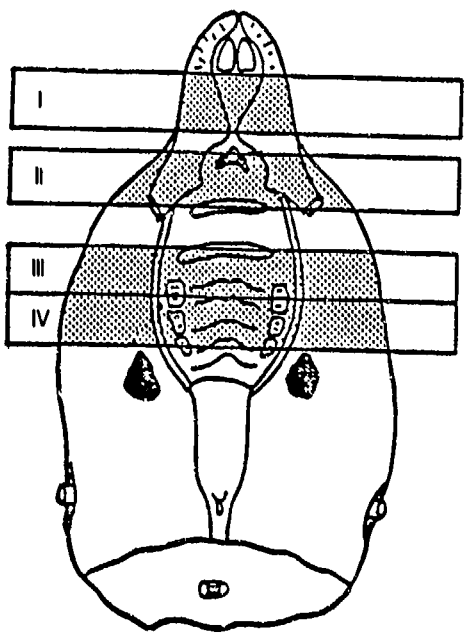

FIG. 3. Nasal cavity regions prepared for histopathologic analysis. This figure was adapted from Young (1981).

periterminal bronchiolar alveolar structures, 2 = multifocal appearance of an abnormality, 3 = several affected alveolar structures, and 4 = many to all periterminal alveolar structures demonstrated the abnormality. The relative intensity index ranged from 0 to 4 with 0 = no abnormality, 1 = trace but detectable alterations in the amount of abnormal material, 2 = mild amount or small changes, 3 = moderate amount of abnormal material or abnormal number of cells, and 4 = large amounts of intra-alveolar material or large changes in cell numbers.

Statistical Analyses: Body weight and lung gravimetric data were analyzed for significant differences between groups using a two-tailed t test for unpaired data (Snedecor and Cochran, 1969). The histopathologic data for the different exposure groups were compared using the MannWhitney nonparametric test for unpaired data (Zar, 1984). P values < 0.05 were considered to indicate significant differences between group values for a given endpoint.

RESULTS

Ventilatory Patterns in Pseudo-Mouth Breathing Rats: Preliminary studies conducted to compare the ventilatory parameters of animals breathing through the mouthpiece with the normal ventilatory parameters of nose-breathing animals during inhalation of filtered air indicated slight but significant differences between the two groups in V_E and f, Table 1. On average, the MB rats had ~7% higher V_E values compared to those of the NB group. This difference appeared to be totally attributable to higher breathing frequencies in the MB group. Values of lung resistance (R) and dynamic pulmonary compliance (C_{dyn}) were slightly but significantly lower with anesthetized MB rats compared to values for these parameters measured with the NB controls.

24 Hr. Post-Exposure Mortalities: No post-exposure deaths occurred with NB and MB animals after exposure to air. Approximately 6% of the animals died after nose exposure to HCl, and 8% died after nose exposure to HBr. No deaths occurred with rats exposed to HF. No post-exposure deaths occurred after MB rats were exposed to air. However, 19% of the MB rats died within 24 hrs after exposure to HBr, 25% of rats died within 24 hr after exposure to HF, and 46% died after exposure to HCl.

24 Hr. Post-Exposure Body Weight Changes: Animals in all NB groups, including the air-exposed rats, experienced body weight reductions beginning 1 day after exposure, Figure 4. Compared to the air-exposed group, NB animals exposed to HCl, HBr, or HF experienced

greater body weight losses beginning 24 hr thereafter, Figure 4, with the HCl and HF exposures causing the greatest weight reductions (~ 10% body weight reductions).

Body weight reductions also were observed in MB animals exposed to the halides or air only, Figure 4. In these cases, the reductions in weight following air-exposure only were significantly greater than the weight decreases found with air-exposed NB animals ($p < 0.05$), whereas the body weight reductions following exposure ofthe MB rats to the different halides were generally less than body weight reductions obtained with the halide-exposed NB animals.

24 Hr. Post-Exposure Lung Gravimetric Parameters: No significant differences were found in the LWW or RCLDW of NB animals exposed to the HF or HBr atmospheres when compared to corresponding values obtained from the lungs of air-exposed NB animals, Figures 5 and 6. The LWW values of NB rats exposed to HCl, however, were slightly, but significantly, elevated above the LWW of their air control counterparts, $p< 0.05$. This latter statistical outcome may represent a Type I error in that corresponding significant increases in RCLWW (data not shown) or RCLDW did not follow exposure to the HCl. Both the LWW and RCLDW of MB animals exposed to HF and HCl were significantly increased 24 hrs after exposure relative to values measured for these parameters with MB rats exposed to air only. No differences in the lung gravimetric parameters were found, however, after exposure to the HBr atmosphere. LWW and RCLDW increases for the MB rats exposed to HCl and HF were not significantly different from one another, whereas the LWW and RCLDW values following MB exposure to HCl and HF were generally significantly greater than the corresponding values obtained from the lungs of HBr-exposed MB animals, Figures 5 and 6.

Nasal Histopathology: No abnormalities were found in the nasal passages of NB rats exposed to air only. NB animals exposed to HBr had a severe necrohemorrhagic rhinitis in Region I of the nasal compartment (see Figure 3 for point of reference), which was sometimes bilateral and sometimes much more severe unilaterally. The mucosa and submucosa in this region were necrotic, with necrosis extending to the turbinate bone, Table 2. Thrombosis of vessels and hemorrhage were also prominent features of the injurious response, as was the presence of fibrin and fluid in the nasal passages. Additionally, PMN were present in modest numbers in the submucosa and in the lumen. The other three regions of the upper respiratory tract were essentially spared from such injury, Table 2. NB animals exposed to HCl also had a severe, necrotizing rhinitis in Region I, often with full thickness necrosis of the turbinates, thrombosis of vessels of the nasal submucosa, and fibrinous pseudomembrane formation, Table 2. Infiltrates of PMN in response to the HCl-induced injury were mild to moderate. Unlike with HBr, the response to HCl extended into Region II of the nasal compartment, Table 2, whereas Regions III and IV remained normal in appearance. Similar to the responses with

TABLE 1

PULMONARY FUNCTION VALUES OF NOSE- AND MOUTH-BREATHING RATS DURING A 32-MIN EXPOSURE TO FILTERED AIR

	Pulmonary function values	
	Nose breather	Mouth breather
Minute ventilation (ml/min)	249 ± 1.6	268 ± 2.4*
Tidal volume (ml)	2.32 ± 0.01	2.33 ± 0.02
Frequency (breaths/min)	106 ± 0.6	113 ± 0.5*
Lung resistance (cmH_2O/ml/sec)	0.40 ± 0.007	0.35 ± 0.005*
Pulmonary compliance (ml/cm H_2O)	0.41 ± 0.006	0.37 ± 0.005*

Note. Lung resistance and pulmonary compliance values were measured on a separate group of anaesthetized rats. Each value represents the mean and standard error of the mean of $N = 6$ to 9 rats.

* Significantly different from nose-breathing control rats. $p \leq 0.05$.

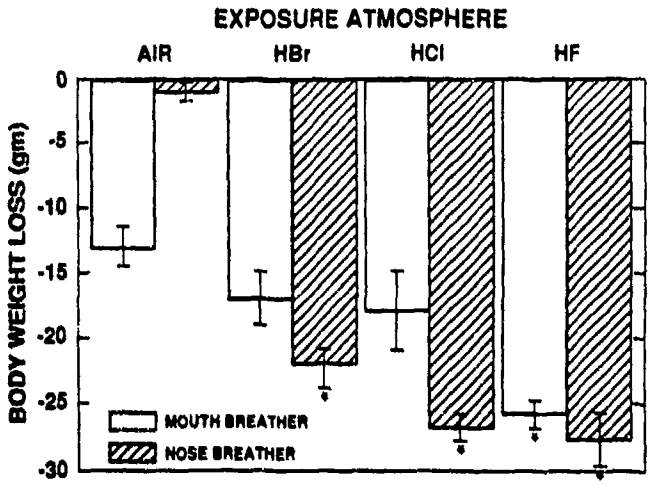

FIG. 4. Body weight reductions of rats 24 hr after exposure via the mouth or nose to either air, 1300 ppm HBr, 1300 ppm HCl, or 1300 ppm HF for a period of 30 min. Each value represents the mean ± SE of data obtained with five to eight rats per group. *Significantly different from body weight reductions found with rats exposed to air via the nose or mouth, $p < 0.05$.

the other halides, the NB animals exposed to HF had a fibrinonecrotic rhinitis that varied from moderate to very severe in nasal Region I, Figure 7A, which was accompanied by large fibrin thrombi in the submucosa and hemorrhage. Although PMN were found enmeshed in fibrin in the nasal passages, relatively few PMN were in the submucosa even in instances of severe necrosis. Instead, PMN were usually most prominent in rats in which the injurious response was less pronounced and necrotizing regeneration of the mucosa was more pronounced. Like the HCl-exposed NB rats, the lesion produced by the inhalation of HF by NB animals extended into Region II, Table 2, whereas Regions III and IV were essentially normal in appearance. Overall, the three halides produced a similar lesion within Region I of the nasal cavity of NB animals with extension of the injury into Region II of the nasal passages being observed after exposure to HF and HCl.

Nasal sections were generally normal after inhalation of air or the halides by the MB rats. However, exudates and/or blood were occasionally found in the turbinates, especially in Regions III and IV. In some instances, Region I also had soft tissue changes in the hard palate mucosa and submucosa, and on the outside of the nares. Such changes were likely due to the procedure used to prepare the rats for the pseudo-mouth-breathing condition.

Tracheal Histopathology: No abnormalities were observed in the trachea of NB rats exposed to air or HBr, Table 3. In a few NB animals exposed to HF and HCl, the trachea showed a mild suppurative inflammatory reaction in the proximal mucosa, which quantitively was not significantly different from the appearance of the tracheae of air-exposed NB animals.

Air-exposed MB animals had a mild-to-moderate fibrinosuppurative tracheitis mainly in the proximal trachea accompanied by neutrophils in the submucosa, Table 4. Although this lesion was generally mild, it was statistically discernible from the relatively normal appearing tracheae of air-exposed NB rats, $p = 0.01$. MB rats exposed to HBr also had a fibrinonecrotic tracheitis which varied in severity; in some sections, fibrin and eosinophilic fluid appeared to nearly occlude the tracheae. The PMN response to HBr was moderate in the tracheal submucosa and lumen and it was quantitatively greater than that observed with the air-exposed MB animals. MB rats exposed to HCl presented a severe, diffuse, ulcerative tracheitis with necrosis of the mucosa and submucosa with fibrin membrane formation covering the ulcerated lumen of the trachea. PMN were observed in the submucosa, between the tracheal rings, and in the connective tissues around the trachea. This latter lesion was also quantitatively greater than the lesion observed in air-exposed MB rats. Epithelial necrosis and the submucosal inflammatory response were greater in MB animals exposed to HCl than in MB animals exposed to HBr. MB rats exposed to HF had a diffuse, very severe fibrinonecrotic tracheitis with moderate influx of PMN in and around the trachea, Figure 7B. The epithelial and

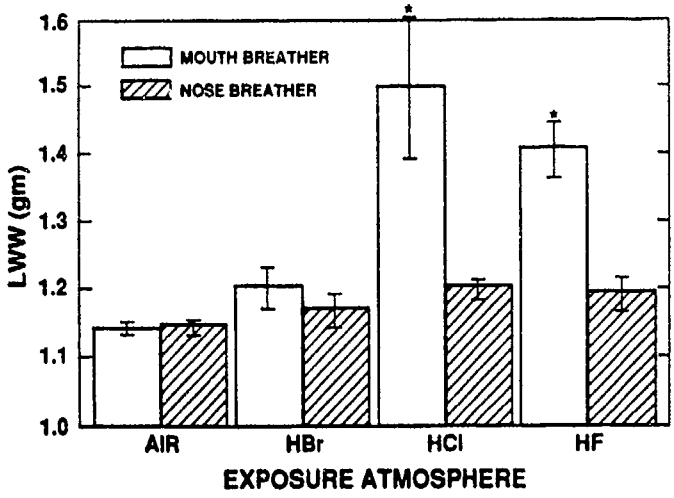

FIG. 5. Lung wet weights (LWW) of rats 24 hr after exposure via the mouth or nose to air or HBr, HCl, or HF for 30 min. Each value represents the mean ± SE of data obtained with five to eight animals per group. *Significantly different from LWW values obtained with rats exposed to air via the nose or mouth, $p < 0.05$.

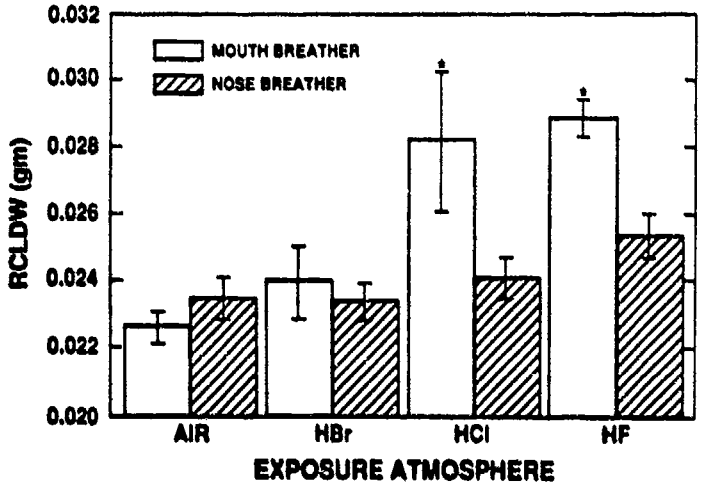

FIG. 6. Right cranial lobe dry weights (RCLDW) of rats 24 hr after exposure via the mouth or nose to air or 1300 ppm HBr, 1300 ppm HCl, or 1300 ppm HF for 30 min. Each value represents the mean ± SE of data obtained with five to eight animals per group. *Significantly different from RCLDW values obtained with rats exposed to air via the nose or mouth. $p < 0.05$.

TABLE 2

HISTOPATHOLOGY OF NASAL CAVITY REGIONS I AND II AFTER EXPOSURE TO HCl, HF, OR HBr VIA THE NOSE

	Nasal section I			Nasal section II		
	HCl	HF	HBr	HCl	HF	HBr
Necrosis						
Epithelial	3.1 ± 0.1*	3.1 ± 0.2*	2.9 ± 0.3*	2.0 ± 0.4*	2.0 ± 0.3*	0.9 ± 0.4
	(3.8 ± 0.1)*	(3.3 ± 0.1)*	(3.3 ± 0.3)*	(2.1 ± 0.4)*	(2.3 ± 0.3)*	(1.0 ± 0.5)
Submucosal	2.9 ± 0.1*	2.5 ± 0.4*	2.6 ± 0.5*	0.4 ± 0.4	0.3 ± 0.3	0.0
	(3.0 ± 0.0)*	(2.6 ± 0.5)*	(2.6 ± 0.4)*	(0.3 ± 0.3)	(0.3 ± 0.3)	(0.0)
Bone	2.1 ± 0.4*	0.4 ± 0.3	1.4 ± 0.4*	0.0	0.0	0.0
	(2.4 ± 0.5)*	(0.3 ± 0.2)	(1.6 ± 0.5)*	(0.0)	(0.0)	(0.0)
Gland	2.4 ± 0.4*	1.9 ± 0.4*	1.4 ± 0.5*	0.0	0.0	0.0
	(2.4 ± 0.4)*	(1.8 ± 0.1)*	(1.4 ± 0.5)*	(0.0)	(0.0)	(0.0)
Exudate						
Protein	1.2 ± 0.6	0.3 ± 0.3	0.4 ± 0.3	0.9 ± 0.4	0.0	0.9 ± 0.5
	(1.4 ± 0.7)	(0.1 ± 0.1)	(0.6 ± 0.4)	(1.3 ± 0.6)	(0.0)	(0.7 ± 0.4)
Fibrin	1.9 ± 0.3*	2.5 ± 0.2*	2.4 ± 0.2*	0.9 ± 0.3*	1.3 ± 0.4*	0.3 ± 0.3
	(2.3 ± 0.2)*	(2.8 ± 0.2)*	(2.6 ± 0.3)*	(1.1 ± 0.5)*	(1.1 ± 0.3)*	(0.1 ± 0.1)
Neutrophils	2.6 ± 0.2*	2.3 ± 0.4*	2.4 ± 0.2*	1.0 ± 0.4*	1.1 ± 0.4*	0.9 ± 0.4
	(2.1 ± 0.1)*	(2.0 ± 0.3)*	(1.9 ± 0.1)*	(1.4 ± 0.5)*	(1.0 ± 0.3)*	(0.4 ± 0.2)
Submucosal Inflammation						
Red blood cells	2.3 ± 0.2*	1.6 ± 0.5*	2.1 ± 0.1*	0.7 ± 0.5	0.0	0.0
	(1.7 ± 0.2)*	(1.3 ± 0.4)*	(1.9 ± 0.1)*	(0.4 ± 0.3)	(0.0)	(0.0)
Neutrophils	2.0 ± 0.0*	2.0 ± 0.3*	2.0 ± 0.0*	1.7 ± 0.4*	1.8 ± 0.4*	0.3 ± 0.3
	(1.7 ± 0.3)*	(1.5 ± 0.3)*	(1.4 ± 0.2)*	(1.1 ± 0.3)*	(1.4 ± 0.3)*	(0.3 ± 0.3)

Note. Values represent the mean ± SE of scored distribution and (severity) values obtained with eight animals per exposure condition.

* Denotes significant difference from air-exposed NB animals, $p \leq 0.05$.

submucosal necrosis scored in MB animals exposed to HF was greater than that produced in the MB animals after HBr inhalation. Also, HF subjectively appeared to have damaged tracheal rings to a greater extent than did HBr or HCl, but this abnormality was difficult to quantitate.

Lung Histopathology: No abnormalities in the lower respiratory tracts of NB rats were detected following exposure to any of the halides. With the exceptions of the trace appearance of fibrin, red blood cells and increased numbers of AM within alveoli, Table 5, the lungs of the MB animals exposed to filtered air only were otherwise normal. MB rats exposed to HBr had some necrosis of the mucosa of the major bronchus. Some of these animals had PMN in scattered alveoli that ranged from modest to large numbers. Quantitatively, the lung lesion produced by inhalation of HBr via the mouth was not significantly different from values measured in air-exposed MB animals, Table 5. MB rats exposed to HCl also had some necrosis of the larger bronchi with PMN infiltrations present in the submucosa, but this lesion was statistically not significantly greater than that present in the air-exposed MB rats. In the more peripheral conducting airways, no necrosis of the bronchial or bronchiolar epithelium was observed, whereas in some lungs there were significantly elevated numbers of PMN in alveoli surrounding some terminal bronchioles. The lungs of MB rats exposed to HF also had focal areas of necrotizing bronchitis of the major bronchi, which quantitatively was significantly greater than that found in air-exposed MB animals. In the alveolar region, scattered foci of PMN in some alveolar ducts and nearby alveoli were observed, and these were sometimes accompanied by free erythrocytes and small increases in AM. The PMN occasionally filled alveoli, but more usually they were present in modest numbers, Figure 7C. Overall, the patterns and degree of pathologic alterations in the lungs following the inhalation of HF, HBr, and HCl were similar with the main exception being a generally greater abundance of neutrophils in the alveoli of the rats exposed to HCl and HF.

Ventilation during Halide Exposure: The inhalation of HF through the nose caused an abrupt and persistent decrease in minute ventilation, Figure 8. Over the 30-min exposure period, a mean V_E of 159 ± 19 ml/min was measured. This represented an ~27% reduction in V_E compared to that of air-exposed NB rats (219 ± 17 ml/min). This response was attributable to marked reductions in breathing frequency (f) upon the onset of exposure as well as to decreases in tidal volume (V_T) over the course of the exposure period, Figures 9 and 10. A mean f of 88 ± 13 breaths per min was measured during exposure to HF, which represents an average 9% reduction from the 97 ± 5 breaths per minute measured during exposure to air only. The V_T of HF-exposed rats gradually fell over the course of exposure, resulting in a

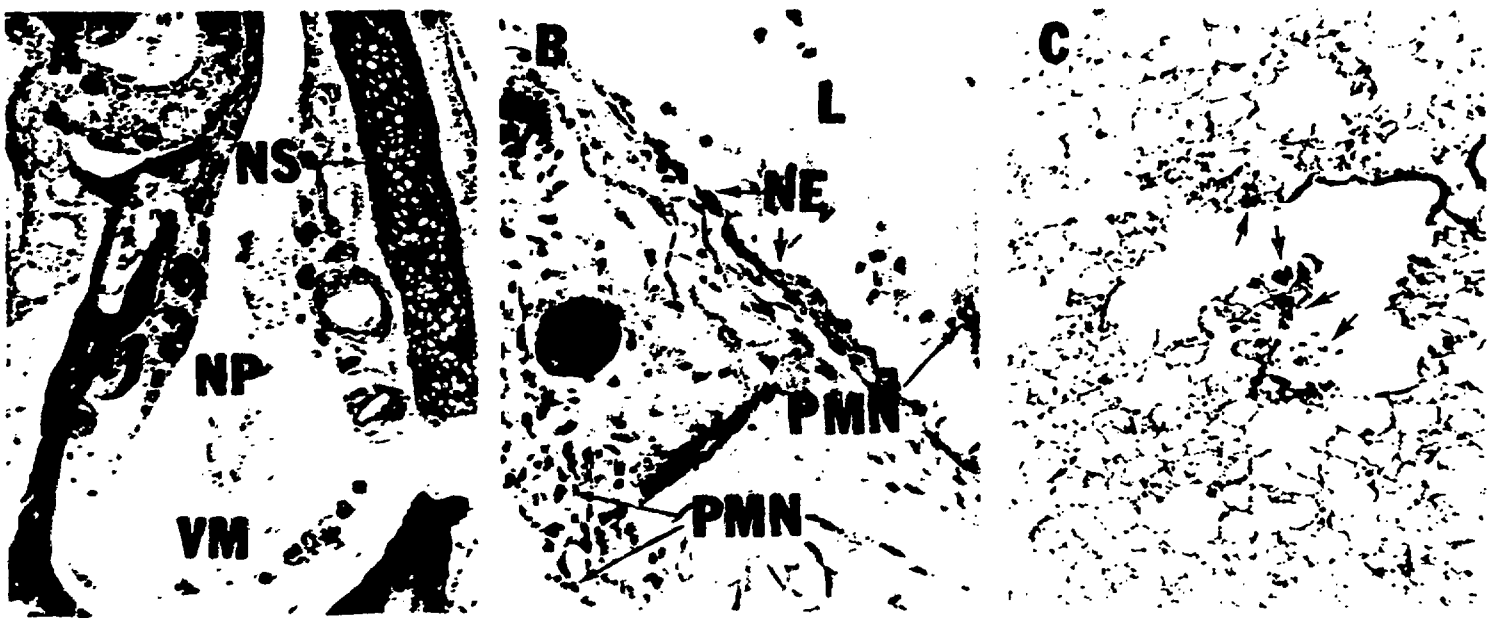

FIG. 7. (A) Micrograph of injury in nasal Region I of an NB rat that was exposed to HF. Severe diffuse necrosis of the epithelium and underlying connective tissues was observed, which was accompanied by fibrin, fluid, and inflammatory cells in the nasal passage (NP). VM: ventral meatus. NS: nasal septum. (B) Micrograph of the trachea of an MB rat that was exposed to HF. Extensive necrosis was observed in the epithelium (NE), the underlying connective tissues, and tracheal cartilages. Polymorphonuclear leukocytes were observed in eosinophilic fluid present in the tracheal lumen (L), as well as in the connective tissue surrounding the trachea. (C) Micrograph of the alveolar region of a MB rat that was exposed to HF. Aggregates of polymorphonuclear leukocytes were observed in the alveolar ducts and nearby alveoli (arrows).

TABLE 3

HISTOPATHOLOGY OF THE TRACHEA OF ANIMALS EXPOSED TO AIR, HCl, HF, OR HBr VIA THE NOSE

	Air	HCl	HF	HBr
Necrosis				
Epithelial	0.0	0.4 ± 0.4	0.3 ± 0.3	0.0
	(0.0)	(0.6 ± 0.6)	(0.3 ± 0.3)	(0.0)
Submucosal	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)
Cartilage	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)
Exudate				
Protein	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)
Fibrin	0.0	0.4 ± 0.4	0.0	0.0
	(0.0)	(0.4 ± 0.4)	(0.0)	(0.0)
Neutrophils	0.0	0.4 ± 0.4	0.0	0.0
	(0.0)	(0.4 ± 0.4)	(0.0)	(0.0)
Submucosal inflammation				
Neutrophils	0.0	0.8 ± 0.6	0.3 ± 0.3	0.0
	(0.0)	(0.7 ± 0.5)	(0.3 ± 0.3)	(0.0)

Note. Values represent the mean and SE of scored distribution and (severity) values obtained with eight animals per exposure condition.

* Denotes significant difference from air-exposed NB animals, $p \leq 0.05$.

TABLE 4

HISTOPATHOLOGY OF THE TRACHEA OF ANIMALS EXPOSED TO AIR, HCl, HF, OR HBr VIA THE MOUTH

	Air	HCl	HF	HBr
Necrosis				
Epithelial	1.5 ± 0.2	3.1 ± 0.5*	3.6 ± 0.2*	2.6 ± 0.2*
	(1.7 ± 0.2)	(3.4 ± 0.4)*	(4.0 ± 0.0)*	(2.7 ± 0.2)*
Submucosal	0.0	2.0 ± 0.5*	3.0 ± 0.3*	1.2 ± 0.4*
	(0.0)	(2.0 ± 0.6)*	(2.9 ± 0.1)*	(1.3 ± 0.4)*
Cartilage	0.0	0.0	1.6 ± 0.3*	0.9 ± 0.4
	(0.0)	(0.0)	(2.0 ± 0.3)*	(1.1 ± 0.4)
Exudate				
Protein	0.0	0.7 ± 0.5	0.0	0.0
	(0.0)	(0.7 ± 0.5)	(0.0)	(0.0)
Fibrin	1.7 ± 0.2	2.9 ± 0.4*	2.8 ± 0.3*	2.6 ± 0.2*
	(1.7 ± 0.2)	(2.7 ± 0.4)*	(3.3 ± 0.3)*	(2.2 ± 0.1)*
Neutrophils	1.7 ± 0.2	2.6 ± 0.4*	2.4 ± 0.3*	2.6 ± 0.2*
	(1.7 ± 0.2)	(2.1 ± 0.3)	(2.6 ± 0.2)*	(2.0 ± 0.0)
Submucosal inflammation				
Neutrophils	1.7 ± 0.2	3.0 ± 0.2*	2.4 ± 0.3*	2.0 ± 0.3
	(1.3 ± 0.2)	(2.7 ± 0.3)*	(2.3 ± 0.2)*	(1.8 ± 0.2)*

Note. Values represent the mean ± SE of scored distribution and (severity) values obtained with 7 to 15 animals per exposure condition.

* Denotes significant difference from air-exposed MB animals, $p \leq 0.05$.

TABLE 5

HISTOPATHOLOGY OF THE LUNG OF ANIMALS EXPOSED TO AIR, HCl, HF, OR HBr VIA THE MOUTH

	Air	HCl	HF	HBr
Alveoli				
Fibrin	0.1 ± 0.1	0.6 ± 0.3	0.0	0.0
	(0.1 ± 0.1)	(0.7 ± 0.4)	(0.0)	(0.0)
	[0.1 ± 0.1]	[0.6 ± 0.3]	[0.0]	[0.0]
Neutrophils	0.0	0.9 ± 0.3*	1.5 ± 0.5*	0.8 ± 0.4
	(0.0)	(1.3 ± 0.5)*	(1.5 ± 0.5)*	(0.8 ± 0.4)
	[0.0]	[1.4 ± 0.6]*	[1.3 ± 0.4]*	[0.8 ± 0.4]
Red blood cells	0.6 ± 0.2	1.0 ± 0.4	0.2 ± 0.2	0.3 ± 0.2
	(0.9 ± 0.3)	(1.4 ± 0.5)	(0.2 ± 0.2)	(0.8 ± 0.4)
	[0.9 ± 0.3]	[1.4 ± 0.5]	[0.2 ± 0.2]	[0.6 ± 0.3]
Macrophages	0.4 ± 0.2	0.8 ± 0.3	1.0 ± 0.4	0.5 ± 0.2
	(0.7 ± 0.3)	(1.0 ± 0.4)	(0.8 ± 0.4)	(1.0 ± 0.5)
	[0.3 ± 0.1]	[0.7 ± 0.3]	[0.5 ± 0.2]	[0.7 ± 0.3]
Type II hyperplasia	0.2 ± 0.1	0.3 ± 0.3	0.5 ± 0.3	0.0
	(0.3 ± 0.2)	(0.3 ± 0.3)	(0.5 ± 0.3)	(0.0)
	[0.3 ± 0.2]	[0.1 ± 0.1]	[0.4 ± 0.3]	[0.0]
Bronchi				
Epithelial	0.0	1.5 ± 0.8	1.1 ± 0.2*	0.5 ± 0.2
Necrosis	(0.0)	(1.7 ± 0.8)	(2.8 ± 0.3)*	(0.7 ± 0.3)
Submucosal	0.0	0.4 ± 0.4	0.0	0.0
Necrosis	(0.0)	(0.4 ± 0.4)	(0.0)	(0.0)
Submucosal	0.0	0.8 ± 0.6	0.6 ± 0.3	0.0
Neutrophils	(0.0)	(0.9 ± 0.6)	(0.7 ± 0.4)	(0.0)

Note. Values represent the mean ± SE of scored distribution. (severity) and [intensity] values obtained with 7 to 15 animals per exposure condition.

* Denotes significant difference from air-exposed MB animals, $p < 0.05$.

mean V_T of 1.84 ± 0.19 ml for an average 19% reduction from the 2.29 ± 0.13 ml measured in air-exposed animals.

The ventilatory changes recorded during inhalation of HBr were very similar to those found during HF exposure. Mean ventilatory values for V_E, f, and V_T during HBr exposure were 164 ± 25 ml/min, 92 ± 11 breaths/min, and 1.85 ± 0.32 ml, respectively, which correspond to an ~25% reduction in V_E, an ~5% reduction in f, and an ~ 19% reduction in V_T relative to corresponding control values. NB animals exposed to HCl also experienced an abrupt decrease in V_E, but the pattern of change thereafter differed from that observed with the other halides. With the HCl, V_E returned to control levels within minutes after the initial reduction, but then gradually decreased during the remaining exposure period, Figure 8. Both the initial and later decreases in V_E were primarily related to decreases in V_T, Figures 8 and 9. Mean exposure values during HCl exposure for V_E, f, and V_T were 207 ± 35 ml/min, 101 ± 9 breaths/min, and 2.13 ± 0.33 ml, respectively. These values correspond to an overall ~6% reduction in V_E, a 4% increase in f, and an ~7% reduction of V_T compared to values obtained with the air-exposed control group of animals.

Air-exposed MB animals had slightly higher (~7%) mean V_E of 235 ± 21 ml/min compared to that of air-exposed NB animals (219 ± 17 ml/min), Figure 8. However, air-exposed MB animals exhibited an ~23% higher f (120 ± 6 breaths/min) compared to values obtained with air-exposed NB animals (97 ± 5 breaths/ min), Figure 9, and an ~16% lower V_T (1.92 ± 0.17 ml) than values obtained from air-exposed NB animals (2.29 ± 0.13 ml), Figure 10. Inhalation of HF by MB caused an initial rapid increase in V_E followed by a persistent reduction in V_E, Figure 8. Overall, a 14% reduction of V_E was measured during exposure to HF (201 ± 27 ml/min) compared to the V_E measured during MB exposure to air. The reduction in V_E during MB exposure to HF, accordingly, was not as dramatic as that observed with the nose breathers. The HF-associated reduction in V_E with the MB rats was due to an ~ 13% decrease in f (105 ± 9 breaths/min) relative to the f measured with the air-exposed MB animals (120 ± 6 breaths/min), Figures 9 and 10.

Inhalation of HBr by the oral route had no apparent effect on V_E or breathing patterns. Mean values of V_E, f, and V_T measured during MB HBr exposure were 228 ± 26 ml/min, 121 ± 7 breaths/min, and 1.85 ± 0.14 ml, respectively; these values were virtually identical to those obtained with MB animals exposed to air only. MB rats exposed to HCl exhibited an ~8% increase in V_E (255 ± 27 ml/min) over that of MB air-exposed animals (235 ± 21 ml/ min), which was primarily due to an ~10% increase in V_T of 2.13 ± 0.11 ml compared to the V_T of 1.92 ± 0.17 ml measured with the MB air-exposed group, Figure 9.

In summary, rats that inhaled the halide gases via the nasal route experienced greater reductions of V_E compared to their air-exposed controls, amounting to ~6, ~27, and ~25%

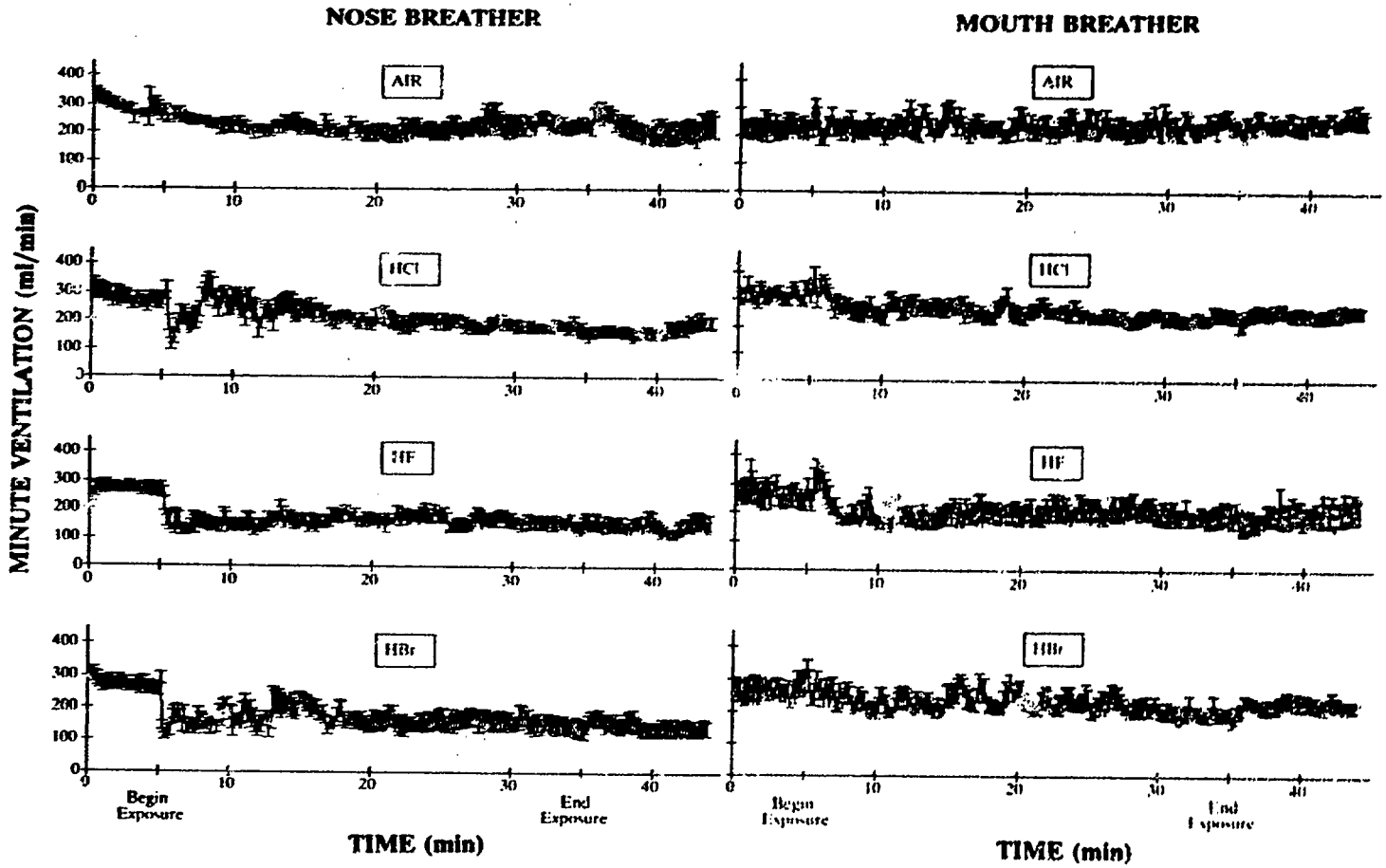

FIG. 8. Minute ventilation ($\dot{V}_E$) values of NB and MB rats during exposure to air and the halides. The curves are the compilation of means and SE of values calculated every 10 sec for all breaths during that exposure period. N = 5–8 rats.

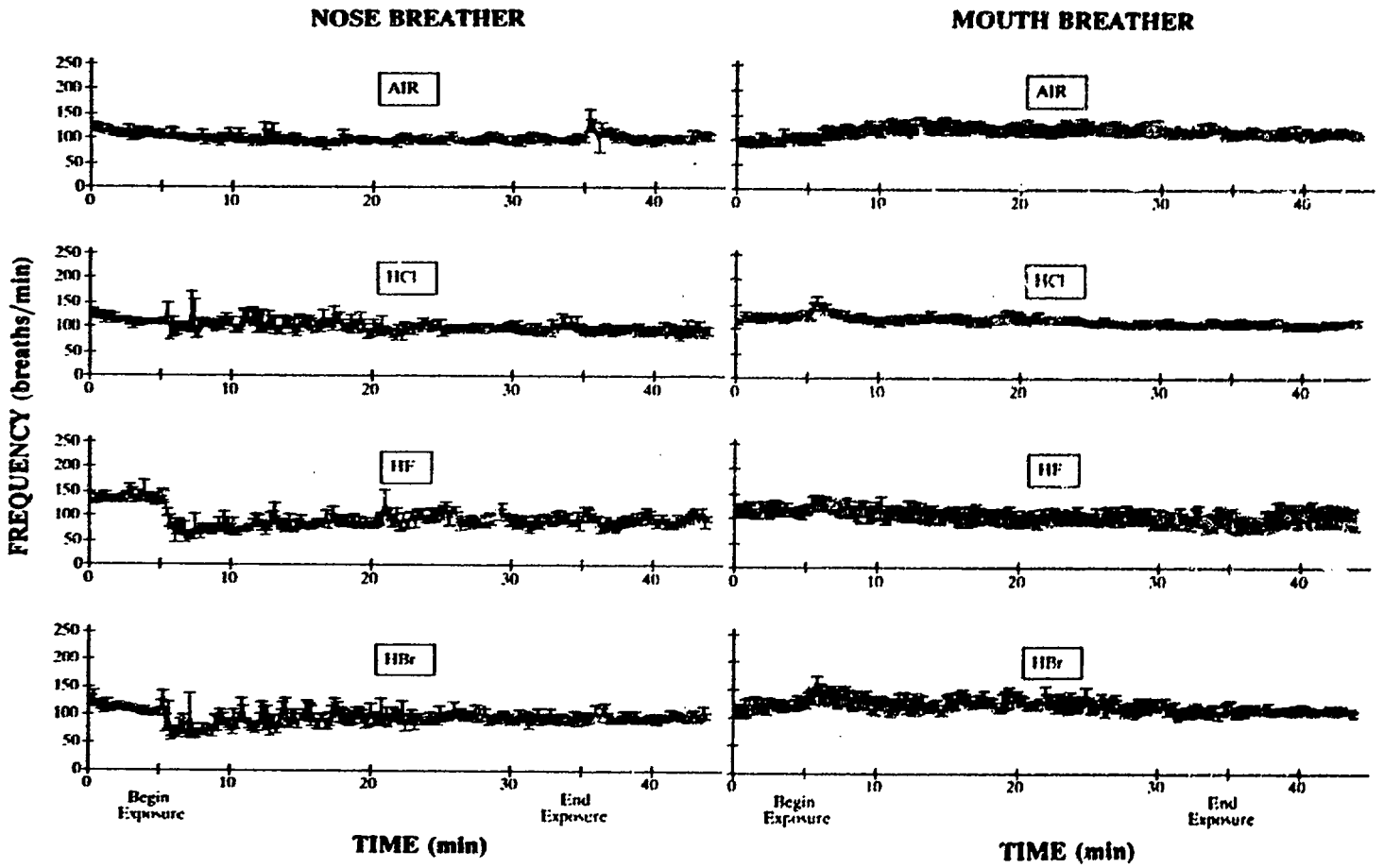

FIG. 9. Breathing frequencies (f) of NB and MB rats during exposure to air and the halides. The curves are the compilation of means and SE of values calculated every 10 sec for all breaths during that exposure period. $N = 5$–8 rats.

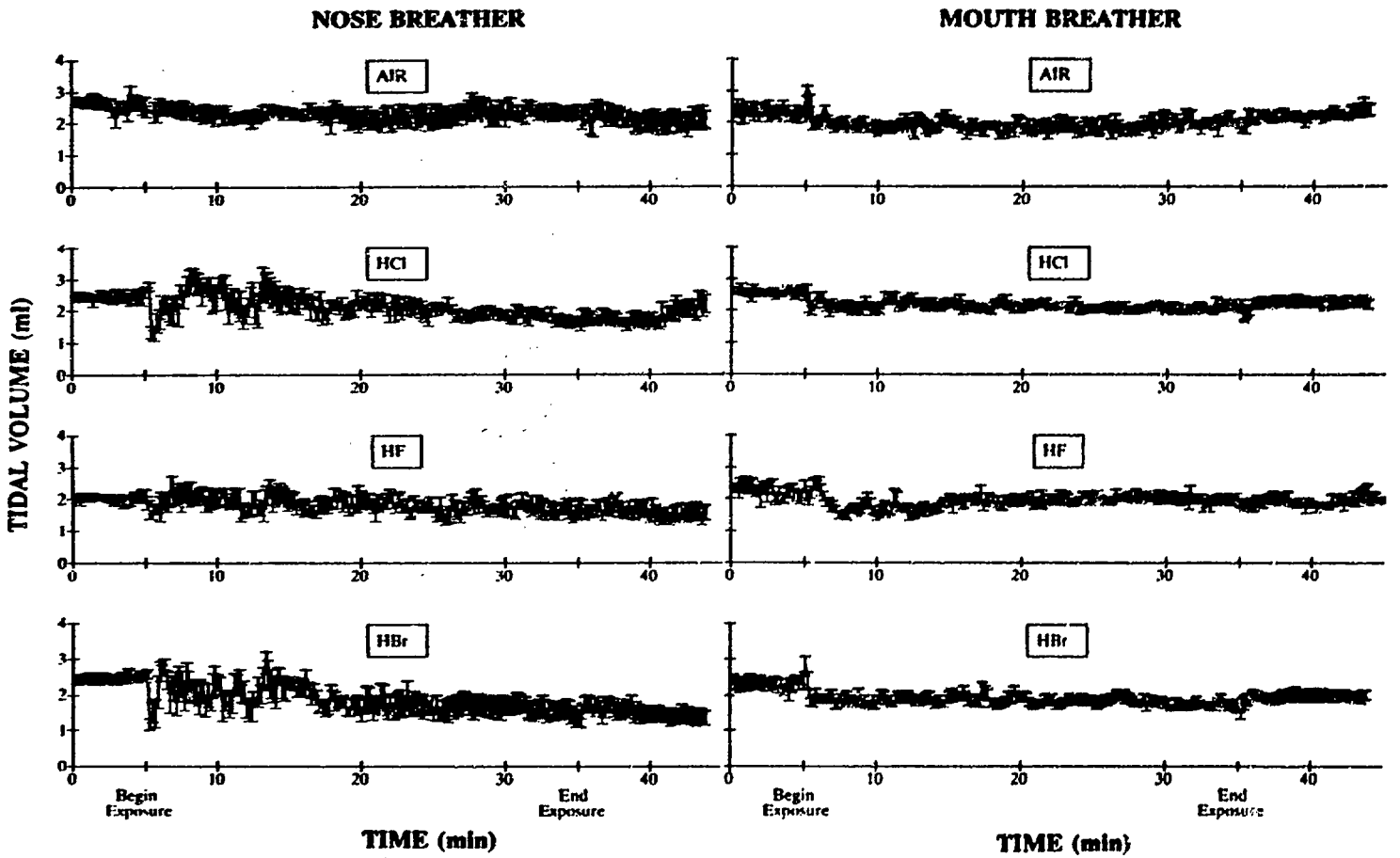

FIG. 10. Tidal volumes (V_t) of NB and MB rats during exposure to air and the halides. The curves are the compilation of means and SE of values calculated every 10 sec for all breaths during that period. N = 5–8 rats.

reductions in the volumes of the atmospheres breathed during exposure to HCl, HF, and HBr, respectively. With the pseudo-mouth breathing rats, V_E increased overall by ~9% during exposure to HCl relative to that of their control counterparts, whereas V_E decreased ~ 15% during exposure to HF and ~ 3% during exposure to HBr. Based on the differing V_E values of the MB and NB rats exposed to the various halide gases, the MB animals were estimated to have inhaled ~23, ~26, and ~39% more of the HCl, HF, or HBr atmospheres, respectively, compared to the amounts inhaled by the NB animals.

DISCUSSION

Numerous investigators have previously utilized obligatory nose-breathing rodent models to examined respiratory tract injury caused by acute exposures to relatively high concentrations of HF and HCl (e.g., Rosenholtz et al., 1963; Machle et al., 1934; DiPasquale and Davis, 1971; Wohlslagel et al. 1976; Morris, 1979). In some earlier studies, upper respiratory tract injury as well as more peripheral pulmonary edema and alveolar hemorrhage followed the exposures (Machle et al., 1934; DiPasquale and Davis, 1971; Wohlslagel et al., 1976), whereas in other more recent investigations, injury to the respiratory tract was confined to the nasal region (Morris, 1979). To our knowledge, similar types of studies have not been conducted with gaseous HBr, even though this halide, like HF and HCl, may be encountered in a variety of environmental settings. In the present study, we re-examined the profiles of injury in the respiratory tracts of obligatory nose-breathing, SPF rats following acute exposures to HF, HCl, and HBr in order to address the above discrepancies in the previously noted pathologic effects of HF and HCl when inhaled through the nasal route and to obtain information on the toxicity of HBr in the respiratory tract as we also attempted to assess the relative toxicities of all three of the halides when breathed through the nose.

Similar to the findings of Morris (1979), we have found that injury to the respiratory tract following the acute inhalation of HF via the nasal route is confined to the nasal compartment; no lung gravimetric or tracheal or lung pathologic evidence was obtained to indicate that HF-induced injury occurred more distally. Moreover, we have found that such nasal injury is restricted to the more anterior regions of the nasal passages. Thus, even the more posteriorly located olfactory epithelial cells, a cell type that has been reported to be especially sensitive to chemical injury (Giddens and Fairchild, 1972; Miller et al., 1981; Buckley et al., 1983; Appelman et al., 1982), were spared from the injurious effects of HF. While our evidence that nasal breathing of a high concentration of HF does not result in demonstrable lung injury per se is inconsistent with the findings of some other investigators (Machle et al., 1934; DiPasquale and Davis, 1971; Wohlslagel et al., 1976), it is consistent with the results obtained by other

investigators (Morris, 1979) as well as with the more recent demonstration that HF is virtually totally absorbed in the nose of the rat (Morris and Smith, 1982). Following the exposure of nose-breathing animals to the other halides, we observed a pattern of respiratory tract injury similar to that caused by HF in that the injurious response primarily occurred in the more anterior nasal passages. The main exception to these outcomes was that some evidence was obtained which suggests that the absorption efficiency of HCl in the nose may be somewhat less than the absorption efficiencies for HF and HBr. Specifically, we did find a slight but significant increase in the LWW of nose-breathing rats after exposure to HCl in addition to some mild pathologic changes that suggested that this halide exerted a toxic effect in the tracheal region. This difference in response, however, may as well be related to the differences in the minute ventilations of the rats exposed to the HF, HCl, and HBr atmospheres, and in turn, to the relative amounts of each halide breathed. Exposure of the animals to HF and HBr had a much more pronounced and essentially identical effect on their minute ventilations while reductions in the minute ventilation of rats exposed to HCl were less dramatic so that the overall minute volumes of the HCl-exposed animals were nearly 30% higher than those of the HF- and HBr-exposed animals during the exposure periods. Taking this into account, our collective findings suggest that gaseous HF, HCl, and HBr are closely equivalent in their toxic effects in the respiratory tract when acutely inhaled at a relatively high mass concentration via the nose.

In another component of our study, we examined the patterns and magnitudes of lower respiratory tract injury when the halides were breathed via the oral route. Such an investigation involving the normally nose-breathing rat required the development of an approach by which human mouth-breathing could be simulated in awake, spontaneously breathing rats. After trying several approaches, the pseudo-mouth-breathing model described herein appeared to be reasonably satisfactory for the present study, even though the model presents some obvious shortcomings. Chief among these is that the placement of the endotracheal tubes often results in the development of local pathologic changes in the trachea that may obscure the actual injurious responses to inhaled materials. Other limitations associated with the pseudo-mouth-breathing rat model are that the ventilatory patterns and at least some pulmonary function parameters, i.e., lung resistance and pulmonary dynamic compliance, are somewhat altered from the nose-breathing condition and that inhaled gases are routed past some anatomical structures of toxicologic interest where local absorption could otherwise take place, e.g., the larynx.

Using the pseudo-mouth-breathing model, we found that the post-exposure mortalities of rats that breathed the halides by way of the oral route were higher than when the HF, HCl, and HBr atmospheres were inhaled by the nasal route. The actual cause(s) of the increased deaths

following exposure to the halides was not determined in the current study. Nevertheless, while the added insult of pathologic changes inherent to the pseudo-mouth-breathing model cannot be ruled out as a contributing factor to such enhancements in mortality, we suspect that the deaths in the mouth-breathing animals resulted from occlusions of the airways secondary to the injurious responses to the halides in the conducting airways atop those stemming from intubation of the animals. Histopathologic evidence of such airway occlusion by fibrin and eosinophilic fluid remained preserved in several longitudinal tracheal sections from halide-exposed rats even after the samples were prepared by infusion of fixative. The fact that we obtained higher mortalities with HCl than with the other halides may not necessarily reflect that this halide is more lethal in the lower respiratory tract then are gaseous HBr and HF. On average, the minute volumes of the mouth breathing rats during exposure to HCl were ~20% higher than the minute volumes of the rats exposed to HF and HBr.

How far the halides penetrated into the lower respiratory tract when inhaled through the oral route was not conclusively determined in our investigation. We did find significant increases in the lung gravimetric parameters following exposure to HF and HCl and we observed a generally focal occurrence of polymorphonuclear leukocytes, erythrocytes, and fibrin in some periterminal bronchiolar alveoli following exposure to all three of the halides. On the other hand, we did not detect any evidence of an alveolar cuboidal cell hyperplasia, i.e., Type II cell hyperplasia, that would suggest Type I pneumocytes were damaged by the halides (Rombout et al. 1986; Warnock, 1982). If the HF, HCl, and HBr are as efficiently absorbed along the conducting airways as they appear to be in the nasal cavity, penetration of these halides to the alveolar region when inhaled via the oral route would not be expected based on available surface areas of the nasal cavity and conducting airways where absorption of the halides from the inhaled air stream can take place. The luminal surface area of the nasal cavity of the adult Fischer 344 rat is 1.3×10^9 μm^2 (Gross et al. 1982). The total surface area of the conducting airways in the lower respiratory tract of the rat is ~5.4×10^9 μm^2 (Yeh et al., 1979), and inhaled gases like the halides can potentially be absorbed by a cumulative surface area equivalent to that in the nasal passages of the rat by the time they pass through airway generations 9 or 10 of the rat's tracheobronchial tree (Yeh et al. 1979). Our failure to find evidence of epithelial damage in the smaller conducting airways following exposure to the halides appears to be consistent with a more proximal absorption of HF, HCl, and HBr. Accordingly, it seems likely that the halide-associated increases in lung weights may have been due to edematous responses in the conducting airways as opposed to more peripheral permeability changes in the alveolar region. Along this same line, the appearance of polymorphonuclear leukocytes and other abnormal constituents in alveoli, which at times essentially filled some alveolar structures, may as well have been a result of airway injury

instead of an alveolar inflammatory response per se. In this regard, the intra-alveolar neutrophils, free erythrocytes, and fibrin in alveoli could have been due to the retrograde passage of these luminal materials from the severely necrotic tracheae as the animals breathed during the post-exposure periods and/or to the mobilization of luminal materials to more distal regions of the lung by the intratracheal instillation of fixative. Further studies, e.g., ultrastructural analyses, are obviously required to determine if the halides actually exert an injurious effect in the alveolar region when inhaled through the oral pathway.

Other experimental findings in our study that we believe merit some comment are the changes in the ventilatory patterns we observed with the nose-breathing and pseudo-mouth-breathing animals while they were exposed to the halides. Similar to our observations, other investigators have previously reported that decreases in breathing frequency and overall decreases in minute ventilation occur in obligatory nose breathing rodents exposed to irritating compounds such as HCl (e.g., Alane,1966; Barlow et al., 1977; Buckley et al., 1984). This response to irritating agents is apparently mediated upon the stimulation of trigeminal nerve endings present in the nasal mucosa (Alane, 1973). In the present study, we have found that ventilatory changes can also occur when the halides are breathed via the oral route and that the ventilatory responses to each of the halides uniquely differed from one another when they are inhaled through this pathway. With HBr, no substantial changes in minute ventilation, breathing frequency, or tidal volume were observed. With HF, on the other hand, the initial ventilatory response was an abrupt, short-lived increase in minute volume due to both increases in breathing rate and tidal volume followed by a persistent reduction in minute volume attributable to a reduced rate of breathing as tidal volume returned the air-exposed condition. With pseudo-mouth-breathing rats breathing HCl, we found slight but detectable increases in minute ventilation, which were associated with an increase in tidal volume over the course of exposure. While the physiologic bases for these responses remain obscure, it remains possible that they reflect differences in the responsiveness of the irritant receptors in the large airways (Barnes, 1986) to the different halides.

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SECTION B

Objective 5: To characterize the upper and lower respiratory tract lesion(s) produced by HCl inhalation in the nose breathing (NB) and mouth breathing (MB) rats during CO_2-induced increased minute ventilation.

Results: Inhalation of 5% CO_2 increased V_E by approximately 70% in NB animals and by approximately 40% in MB animals. The increase in V_E was primarily due to increases in tidal volume (Vt) in both groups of animals. HCl inhalation during NB and MB decreased V_E approximately 45% during these exposures. The primary lesion resulting from NB inhalation of HCl was localized in the most anterior section of the upper respiratory tract and no additional injury was detected due to concurrent CO_2 inhalation. Inhalation of HCl during MB resulted in higher mortality, which was increased with elevated VE during concurrent CO_2 inhalation. Lung gravimetric and histologic parameters indicated that surviving MB animals that were exposed to HCl + CO_2 had greater lower respiratory tract injury compared to MB animals exposed to HCl alone.

Report:

Archuleta, D., Martinez, M., Stavert, D.M., Lehneri, B.E.: Pathologic responses to inhaled HCl during periods of increased minute ventilation in pseudo-mouth breathing and nose breathing rats. 1991 Society of Toxicology Annual Meeting, Dallas TX, February 25-March 1, 1991. The Toxicologist 11(1): A339, 1991.

INTRODUCTION

Gaseous halides can be generated as a pyrolysis products of the fire retardant Halon 1301, as well as from the combustion of other materials. Previous work in our laboratory (Archeleta et al., 1990; Kusewitt et al., 1989; Stavert et al., 1991; Section A) has shown that the respiratory tract injury produced by the acute inhalation of relatively high mass concentrations of HCl, HF, and HBr (~1300 ppm) is localized within the nasal compartment during nose breathing (NB) and to the trachea and higher generation conducting airways during mouth breathing (MB) in the rat. For example, using lung gravimetric and histopathologic criteria, virtually no peripheral lung damage was observed in NB rats, and mild to no lung damage was observed in MB rats 24 hrs after being exposed to 1300 ppm HCl for 30 min. It is presently unknown as to whether or not the lesions induced by acute high level inhalation of the halides can be extended more deeply into the lower respiratory tract by increases in minute ventilation (V_E). The main objective of this study component was to characterize the upper and lower respiratory tract lesions produced by inhaled halide in nose breathing and mouth breathing rats during CO_2 (5%) induced increased minute ventilation with the driving hypothesis being that the inhalation of halide during enhanced minute ventilation will increase the severity of resulting injury while also extending the injurious response to more peripheral regions of the respiratory tract. Inasmuch as we have previously demonstrated that HF, HBr, and HCl are essentially equivalent in producing toxic effects in the respiratory tract (see Section A; Stavert et al., 1991) when inhaled at like concentrations (expressed in ppm), this task was undertaken using HCl as the model for all three of the halides.

METHODS AND MATERIALS

Experimental Design and Approaches: Fischer-344 rats (SPF animals, pre-conditioned to partial body flow plethysmographs) were lightly anaesthetized uniformly with ethrane and either fitted with mouthpieces with attached silastic tracheal tubes (mouth breathers, MB), or they were allowed to awaken without further manipulation (nasal breathers, NB). After recovery, the NB and MB animals were placed into partial body plethysmographs and attached to an exposure chamber. The animals were provided clean filtered air for 5 min while pre-exposure ventilatory parameters were measured. Details about these approaches have been described in Section A.

Exposure atmospheres consisting of either filtered air with or without 5% CO_2 (controls), or 1000 ppm of HCl with or without 5% CO_2, were delivered to the rats for 20

min with the ventilatory parameters being measured over this exposure period. After cessation of the exposures, clean air was delivered to the animals for 10 minutes while post-exposure ventilatory parameters were measured. After the exposures, the mouthpieces and tracheal tubes were removed from the MB animals. MB and NB animals were sacrificed 24 hrs after the exposures for histologic analysis of their upper and lower respiratory tracts and for lung gravimetric measurements.

The mouthpieces were fabricated from the tips of polyethylene centrifuge tubes. The tracheal tube portion of each mouthpiece was made from soft silastic tubing, as described in Section A and illustrated in Figure 1. The device was placed intratracheally into a lightly anethetized rat using a modified otoscope, and it was secured into place via in cisor tooth grip holes drilled into the mouthpiece. A linear flow resistance of 0.11 cm H_2O/ml/sec was obtained with the device up to 20 ml/sec. The device had a deadspace volume of 0.3 ml. These values closely match the nasal resistance and deadspace values found for 250 gm Fischer rats (Stavert and Lehnert, 1988). After the device was in place, a nose clip was attached to the external nares, and a rubber band was placed over the mouth to prevent dislocation of the mouthpiece during exposure.

The HCl exposure atmospheres were generated by mixing pure HCl with anhydrous HEPA filtered air within a stainless steel mixing chamber. CO_2 was added and adjusted to 5% immediately upstream of the exposure chamber. Animals were exposed to the atmospheres while in a flow plethysmograph, Figure 2. A Teflon head piece with 2 rubber dams effectively sealed the body of the animal within the plethysmograph. A neck brace stabilized the animal during exposure. Details about the exposure protocols, monitoring of the exposure atmospheres, and measuring the pulmonary functional status of the rats are given in Section A.

Rat sacrifices were initiated by I.P injections of 50 mg pentobarbital sodium. The nasal cavity regions were prepared as described in Section A, Figure 3. The trachea and lungs were excised, and the heart, extra-pulmonary mediastinal tissue, and the esophagus were removed. The lungs were blotted and weighed (Lung Wet Weight, LWW). The bronchus leading to the right cranial lobe (RCL) was ligated with fine suture, and the RCL was removed and weighed (Right Cranial Lobe Wet Weight, RCLWW). Following the gravimetric measurements, the trachea and lungs, minus the RCL, was cannulated with an 18-ga needle secured with ligature, and the lungs subsequently infused and fixed at a constant pressure of 30 cm H_2O with 10% formalin in phosphate buffered saline. The RCL were oven dried to a constant weight at 100°C for 36 hrs. and reweighed (Right Cranial Lobe Dry Weight, RCLDW).

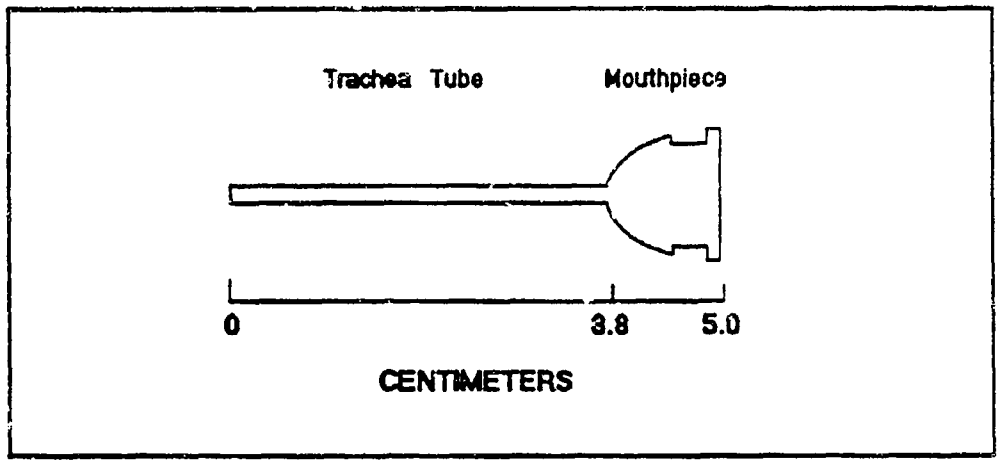

Figure 1. Mouthpiece used by the mouth breathing rats.

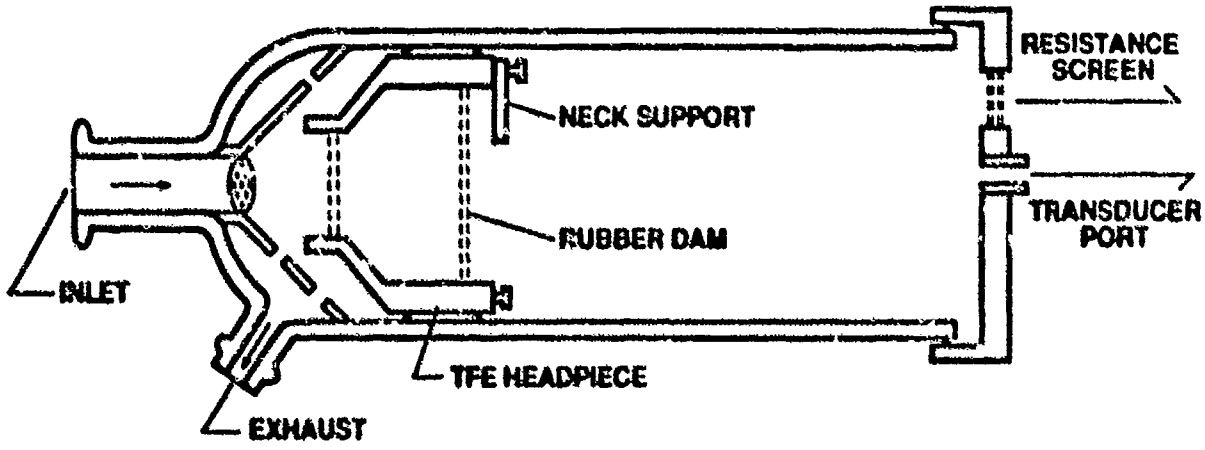

Figure 2. Partial body flow plethysmograph used for the exposures of mouth breathing and nose breathing rats. Rats were trained to sit within the plethysmograph during a 3 day period prior to exposure.

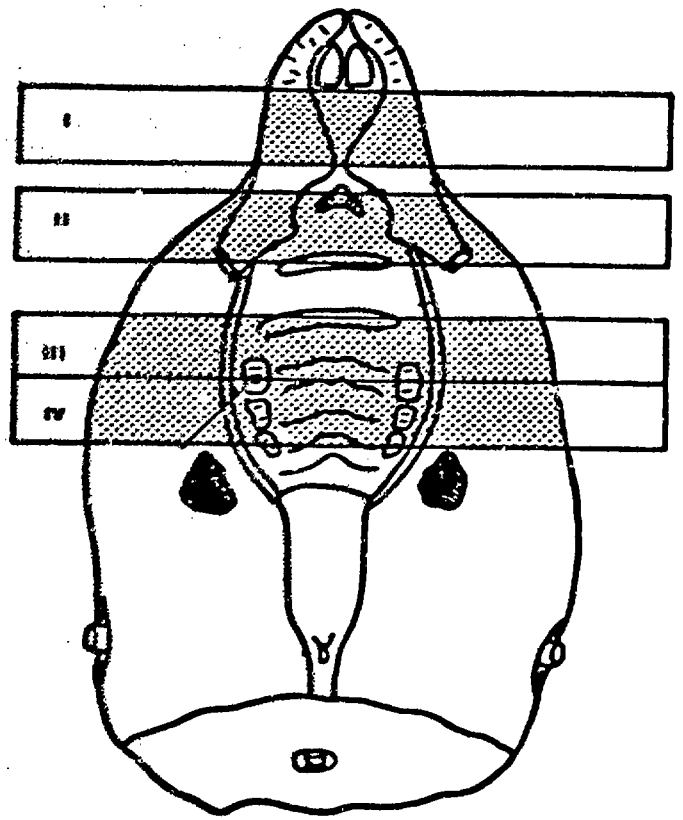

Figure 3. Nasal cavity regions prepared for histopathologic analysis. This figure was adapted from Young, Fund. Appl. Toxicol. 1:309-312, 1981.

Injury in the nasal cavity was graded according to severity and distribution. Endpoints of injury included epithelial necrosis, necrosis of lamina propria structures, theappearance of proteinaceous and cellular exudates, the infiltration of polymorphonuclear leukocytes (PMN) in the epithelium and lamina propria, and hemorrhage into the lamina propria. A grading scale was used to quantitatively index the relative extent or distribution of an abnormality within a given tissue section, as described in Section A. Tracheal and lung injury was also assessed for the endpoints described in Section A.

RESULTS

Ventilatory Responses: Normal NB rats increased V_E approximately 70% during air + 5% CO_2 exposure, while the MB rats increased V_E approximately 36% during the inhalation of air + 5% CO_2, Figure 4. NB rats decreased V_E approximately 20% during 1000 ppm HCl inhalation. Concurrent HCl and 5% CO_2 inhalation by NB rats resulted in no increase in V_E compared to the V_E of HCl-only exposed NB rats, Figure 5. MB rats decreased their V_E approximately 24% during HCl inhalation compared to air exposed MB rats, Figure 6. However, MB rats increased their V_E approximately 45% during 5% CO_2 + HCl inhalation, when compared to MB inhalation of HCl alone. V_E responses during air + CO_2 exposure of NB and MB animals were primarily the result of tidal volume (V_T) changes, Figure 4. While breathing frequency (f) increased during air + CO_2 inhalation in the NB rats, no significant changes in f were measured in the MB rats. Elevation of V_E measured in MB rats during HCl +CO_2 exposure were primarily due to increases in V_T.

Mortalities: No post-exposure mortality was observed in NB animals after exposure to air, air + 5% CO_2, 1000 ppm HCl, or to 1000 ppm HCl + 5% CO_2 atmospheres. No post-exposure deaths occurred after MB rats were exposed to air, or air + 5% CO_2. However, MB rats experienced 40% mortality after exposure to 1000 ppm HCl and 58% mortality after exposure to 1000 ppm HCl + 5% CO_2.

Body Weight Losses: Compared to air exposed controls, NB animals exposed to HCl experienced significant body weight losses (~10% body weight reductions) as of 24 hrs after exposure, Figure 7. HCl + 5% CO_2, which did not increase V_E as noted above, caused no greater body weight reductions compared to the reductions observed after HCl-only exposures. MB animals in all groups, including air controls, experienced 24 hr body weight reductions, Figure 7. Compared to air exposed MB animals, the HCl exposed

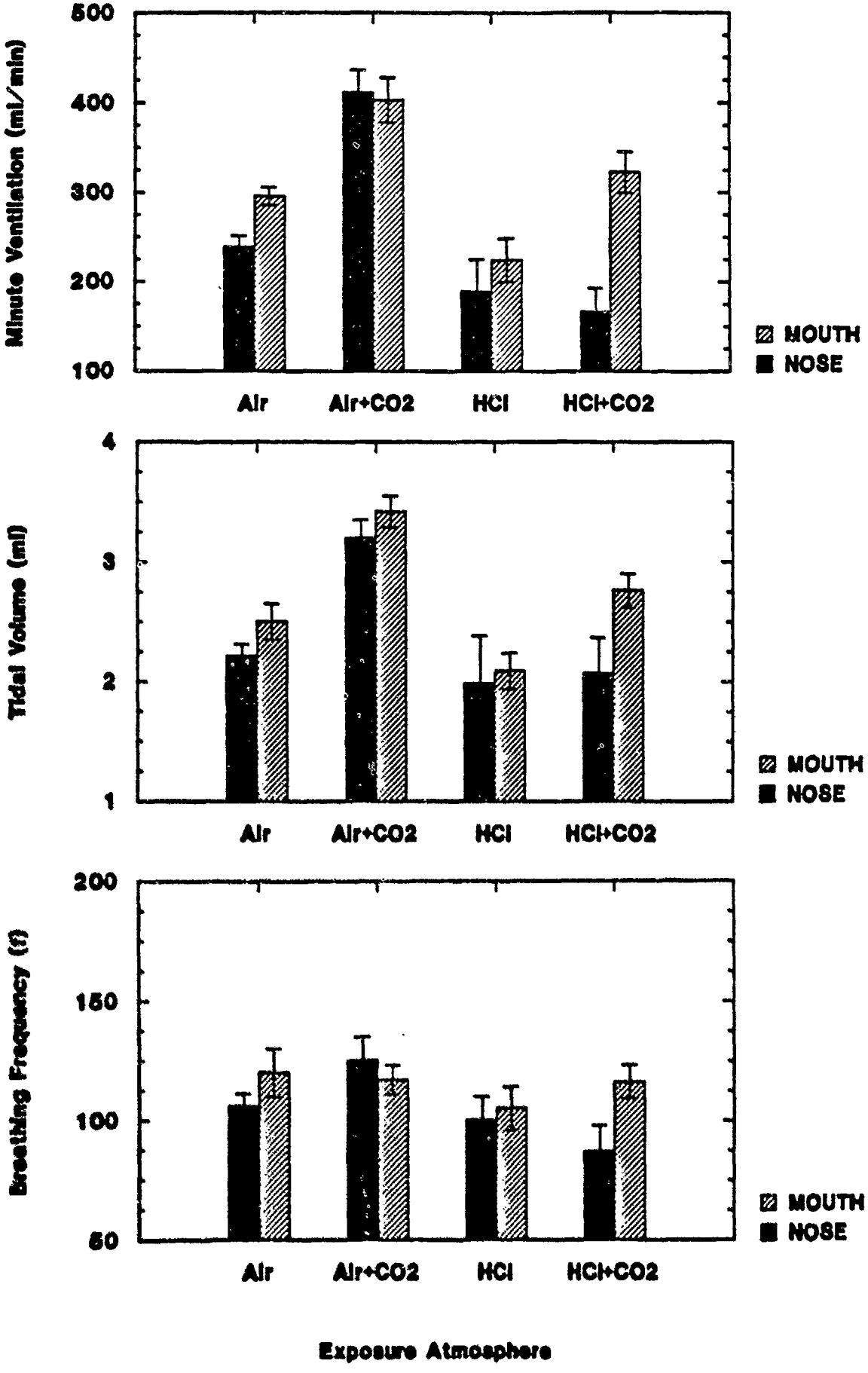

Figure 4. Minute ventilations, tidal volumes and breathing frequencies of rats exposed to air, air + 5% CO_2, 1000 ppm HCl or 1000 ppm HCl + 5% CO_2 via the nose or mouth. Values represent the mean and S.E.M. of n=6 to 12 rats.

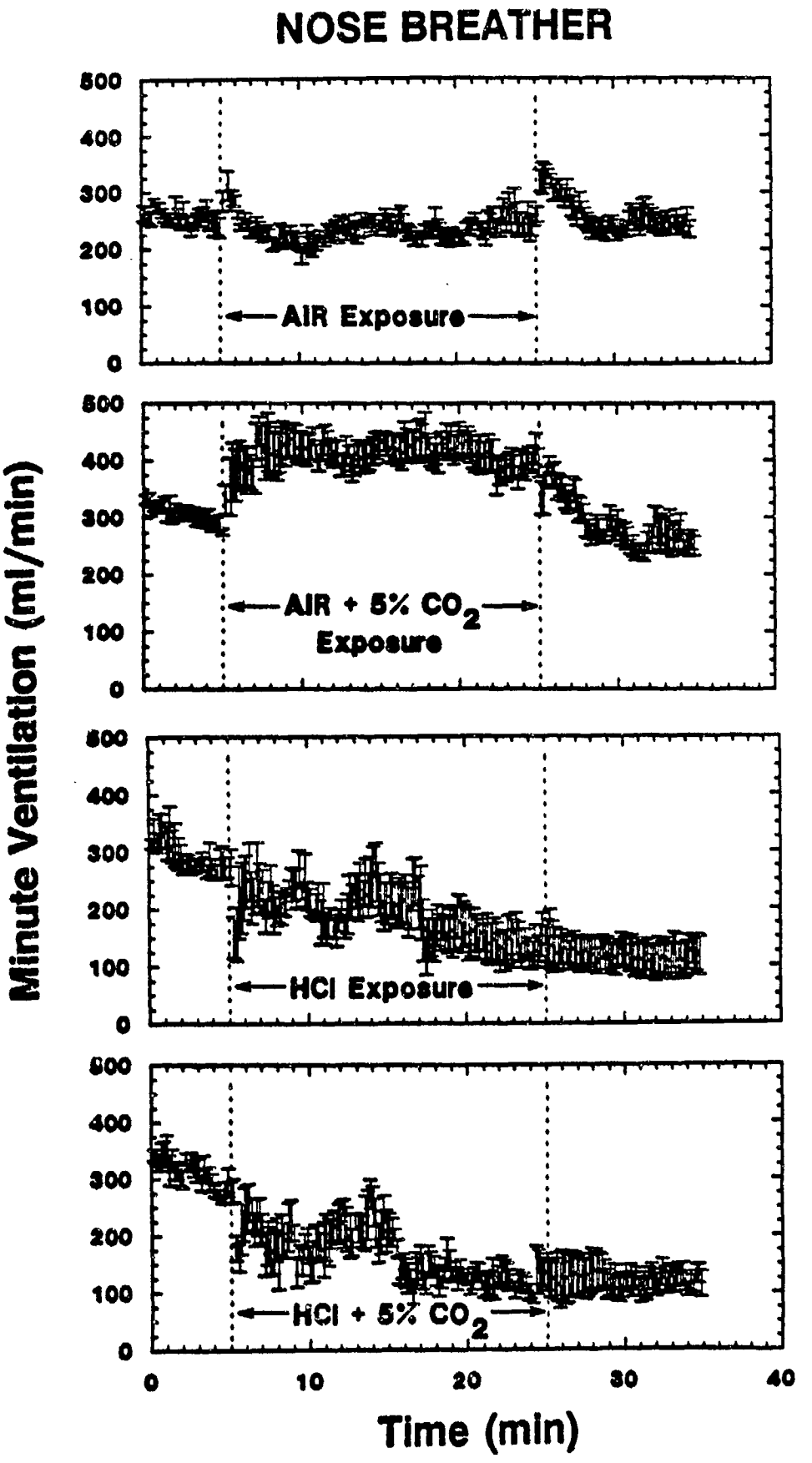

Figure 5. Minute ventilations of nose breathing (NB) rats before, during, and after exposure to air, air + 5% CO_2, 1000 ppm HCl, or 1000 ppm HCl + 5% CO_2. Each point represents the mean and standard error of the mean of average minute ventilation values for a 10 second period of time for n = 6 rats.

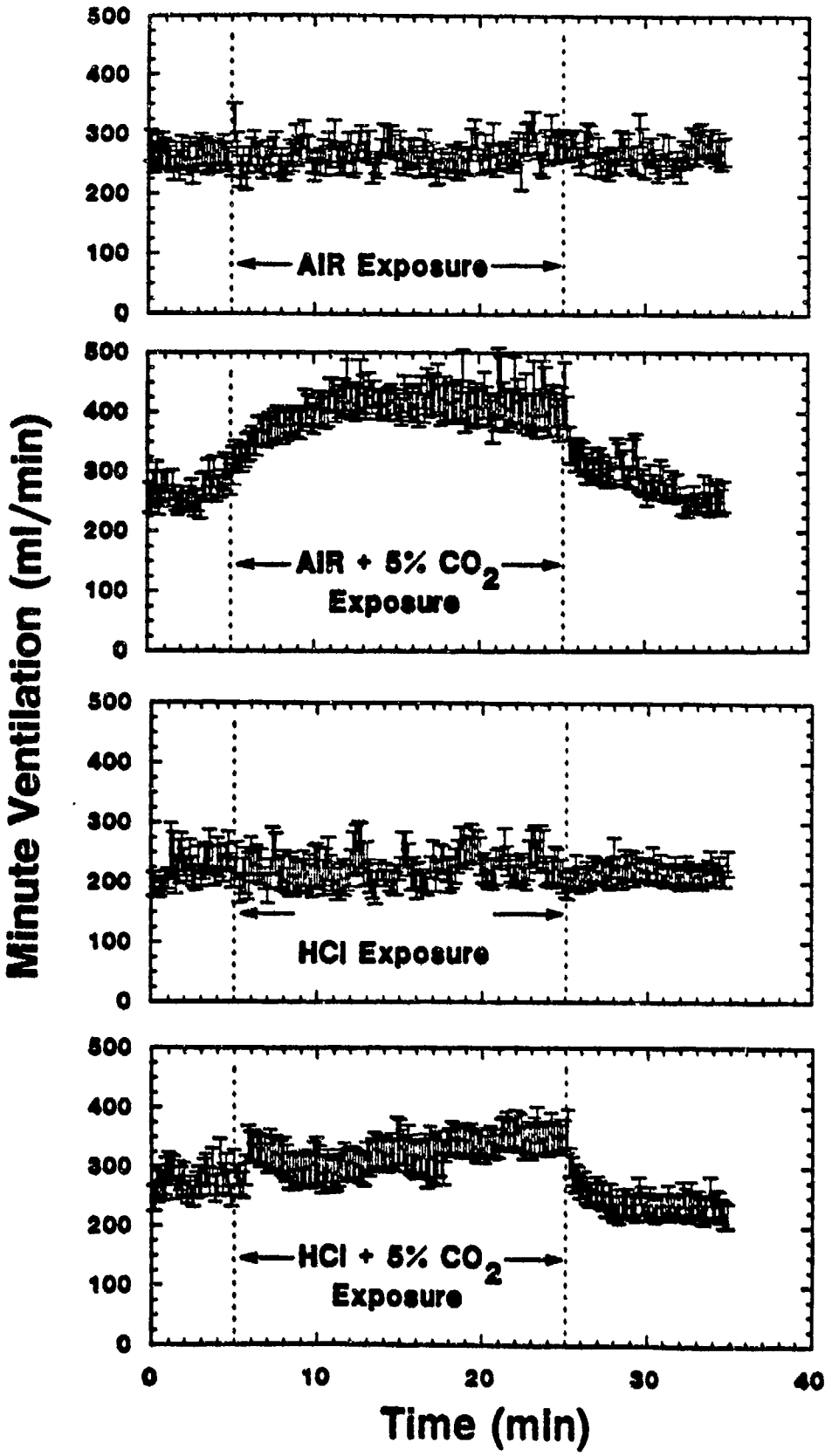

Figure 6. Minute ventilations of mouth breathing (MB) rats before, during, and after exposure to air, air + 5% CO_2, 1000 ppm HCl, or 1000 ppm HCl + 5% CO_2. Each point represents the mean and standard error of the mean of average minute ventilation values for a 10 second period of time for n = 6 to 12 rats.

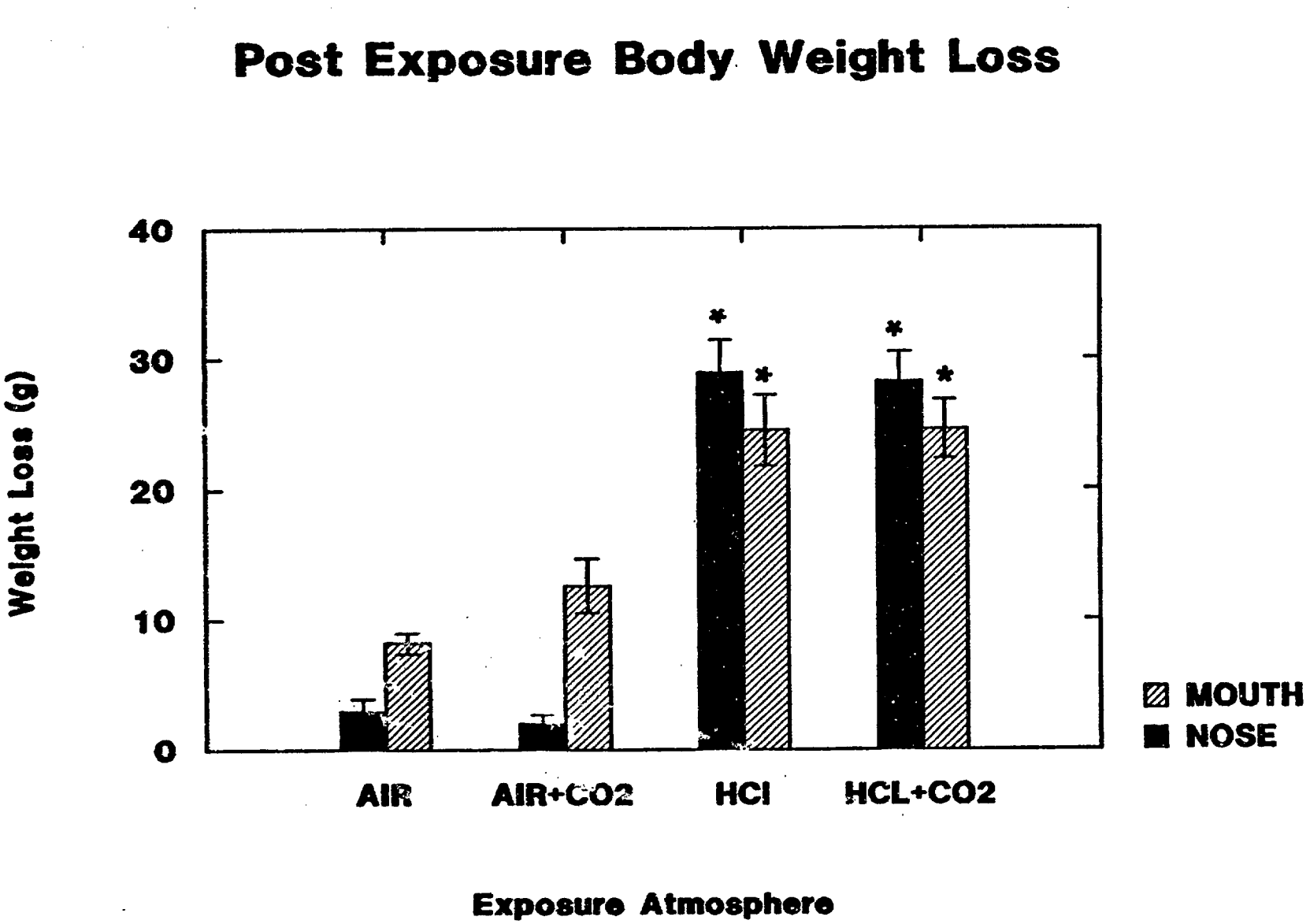

Figure 7. Body weight reductions of rats 24 hr after exposure via the mouth or nose to either air, air + 5% CO_2, 1000 ppm HCl or 1000 ppm HCl + 5% CO_2 for a period of 20 min. Values represent means and standard error of the mean of N = 5 to 6 rats. (*) indicate significant difference compared to body weight reductions found with rats exposed to air via the nose or mouth, $P \leq 0.05$.

animals experienced significantly greater reductions in body weight. The addition of CO_2 with the HCl exposure, which increased V_E as previously indicated, did not affect the body weight loss experienced 24 hrs. after exposure relative to rats exposed to HCl only.

Lung Gravimetric Changes: No significant differences were found in the lung wet weights (LWW) or right cranial lobe dry weights (RCLDW) of NB animals exposed to HCl or HCl + 5% CO_2 when compared to corresponding values obtained from the lungs of air exposed animals, Figures 8 and 9. The LWW, and RCLDW of MB animals exposed to HCl were not significantly different 24 hrs. after exposure compared to air exposed values, Figures 8-9. Significant increases in LWW and RCLDW, however, were measured from animals exposed to HCl + 5% CO_2 compared to values from animals exposed to HCl only.

Histopathology: Air or air + CO_2-exposed NB animals showed no abnormalities in the upper respiratory tract. Animals exposed to HCl had a moderate necrotizing rhinitis in Region I and sometimes Region II of the nasal compartment, see Section A. The necrosis was multifocal with degeneration/regeneration (squamous epithelium) and accompanying exudates in the lumen. The lesion produced upon inhalation of HCl + CO_2 was not discernably different from the lesion in animals exposed to HCl alone. With the MB rats, the nasal sections were basically normal after inhalation of air, HCl, or these atmospheres with CO_2. Exudates or blood was occasionally found in the turbinates, especially in Regions III and IV. Region I sometimes had soft tissue changes in the hard palate mucosa/submucosa and on the outside of the nares.

No lesions occurred within the tracheae of NB rats after exposure to any of the experimental atmospheres. Air exposed MB rats showed a mild fibrino-suppurative tracheitis, mainly in the proximal trachea with neutrophils in the submucosa. MB rats exposed to HCl had a acute necrotizing tracheitis sometimes extending to the submucosa. PMN were observed in the submucosa and in tissues around the trachea with fibrinous pseudomembranes on the inner surface of the trachea. The tracheal injury of rats exposed to HCl + CO_2 was generally more severe, and it extended deeper into the lung. No lesions were found in the lower respiratory tract following NB exposure to air, air + CO_2, HCl, or HCl + CO_2. Air or air + CO_2 exposed MB animals showed no lesion in the lung compartment. The lungs from MB rats exposed to HCl alone were normal in appearance. The lungs of rats exposed to HCl + CO_2 showed necrosis of the major bronchus with PMN present in the submucosa. In the more peripheral conducting airways, there was minor necrosis of the bronchial or bronchiolar epithelium, but in some lungs there were large numbers of neutrophils in alveoli surrounding some terminal bronchioles. Edema

fluid was observed in some rats. A summary of the pathologic lesion location is given in Table 1.

Post Exposure Lung Wet Weight

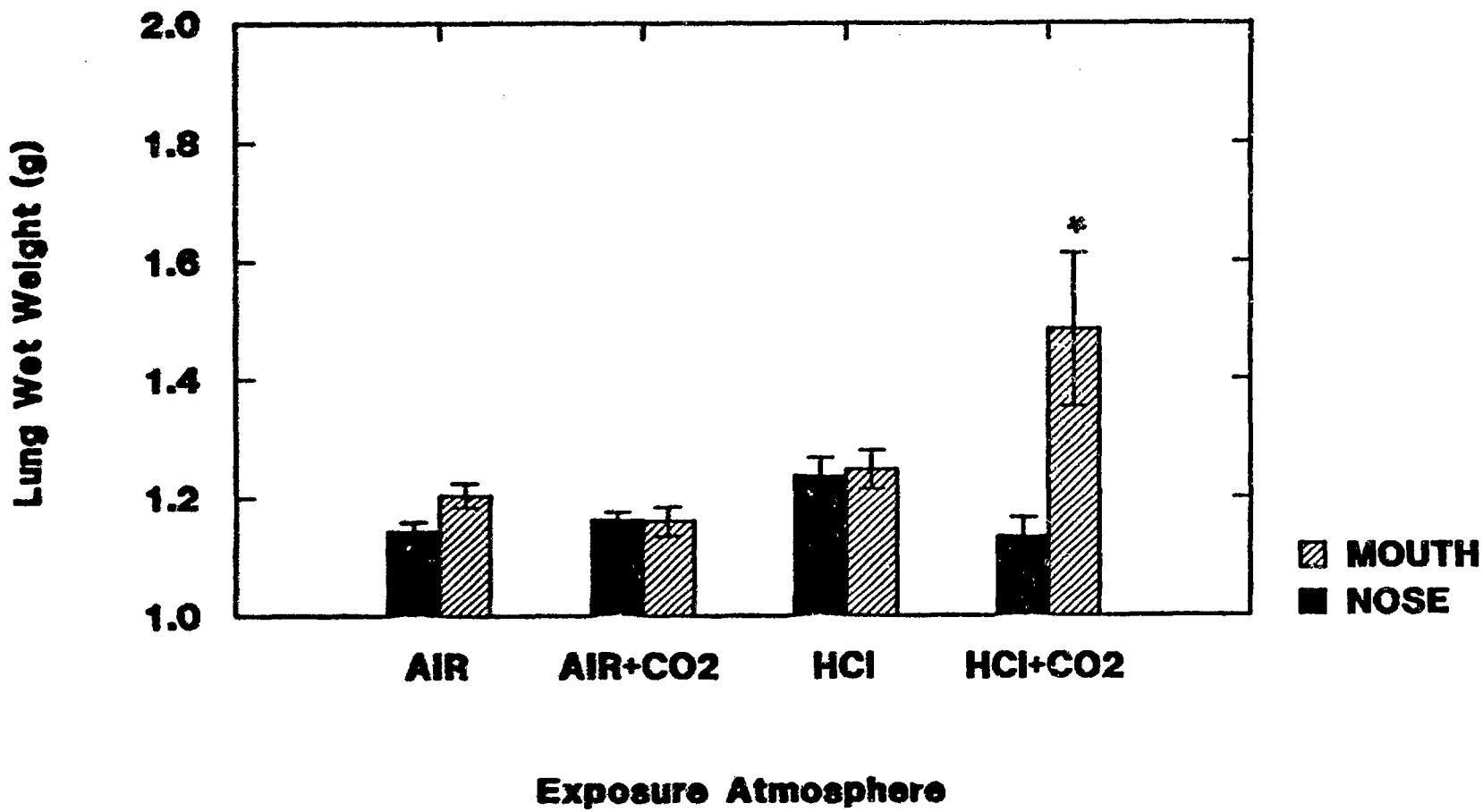

Figure 8. Lung wet weights (LWW) of rats 24 hr after exposure via the mouth or nose to either air, air + 5% CO_2, 1000 ppm HCl, or 1000 ppm HCl + 5% CO_2 for 20 min. Values represent the mean and standard error of the mean of N = 5 to 6 animals. (*) indicate significant difference compared to LWW found with rats exposed to air or HCl only via the mouth, $P \leq 0.05$.

Post Exposure Right Cranial Lobe Dry Weight

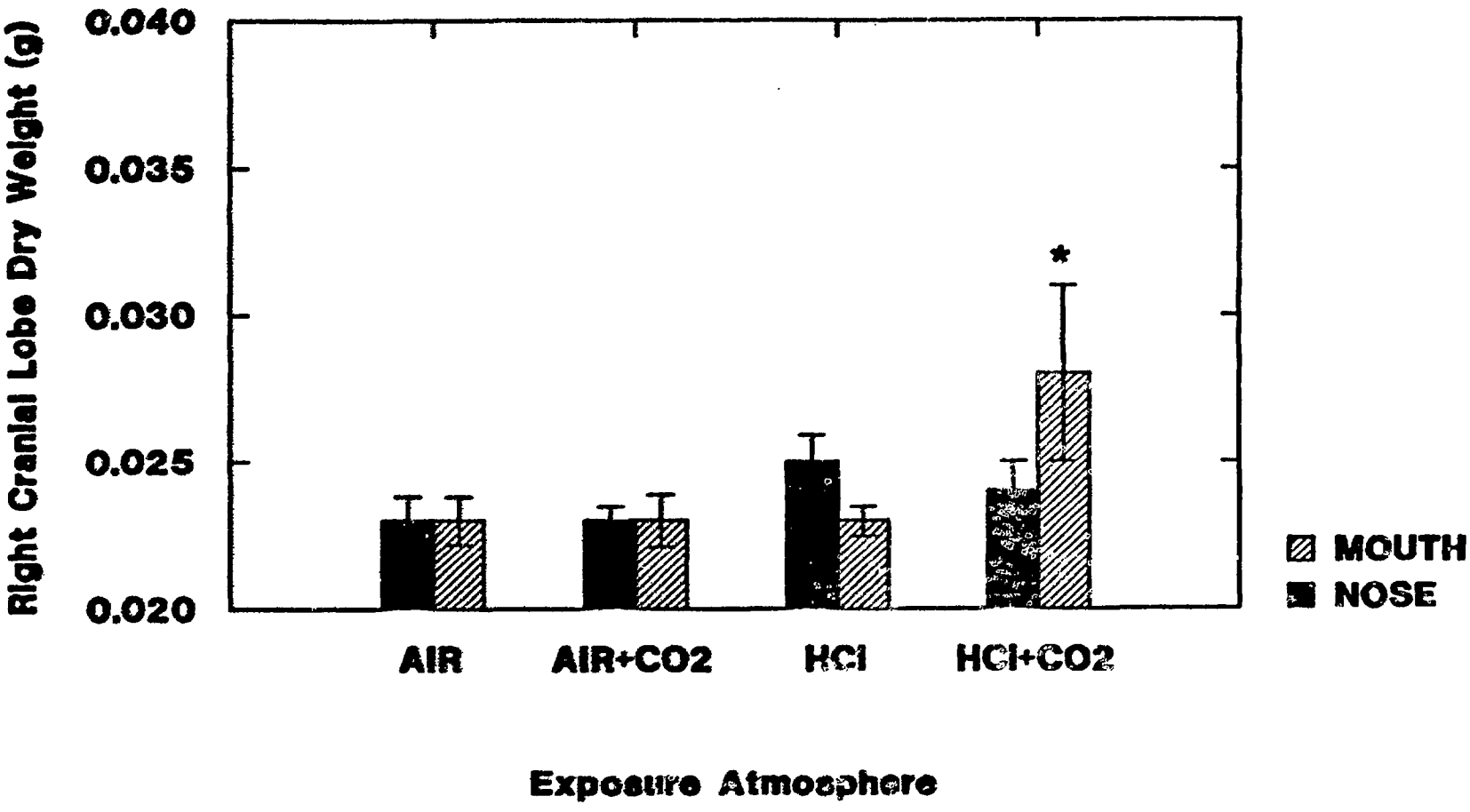

Figure 9. Right cranial lobe dry weight (RCLDW) of rats 24 hr after exposure via the mouth or nose to either air, air + 5% CO_2, 1000 ppm HCl, or 1000 ppm HCl + 5% CO_2 for 20 min. Values represent the mean and standard error of the mean of N = 5 to 6 animals. (*) indicate significant difference compared to RCLDW found with rats exposed to air or HCl only via the mouth, $P \leq 0.05$.

PATHOLOGIC LESION LOCATION						
Exposure Atmosphere	Nasal Section I	Nasal Section II	Nasal Section III	Nasal Section IV	Trachea	Lung
	NOSE BREATHER					
AIR						
AIR + CO_2						
HCl	++	+				
HCl + CO_2	++	+				
	MOUTH BREATHER					
AIR					+	
AIR + CO_2					+	
HCl					++	
HCl + CO_2					+++	++

Table I. Pathologic lesion location 24 hr after acute inhalation of AIR, AIR + CO_2, HCl, HCl + CO_2. (+) indicates mild lesion, (++) indicates relatively moderate lesion, and (+++) indicates severe lesion.

SUMMARY/DISCUSSION

The results from this investigation can be summarized as follows:

• Inhalation of 5% CO_2 increased V_E ~70% in NB animals and ~40% in MB animals. The increase in V_E was primarily due to increases in V_T in NB and MB animals.

• HCl inhalation during NB and MB decreased V_E ~20%. NB animals did not increase V_E during HCl + CO_2 inhalation, while MB animals increased V_E ~45% during these exposures.

• The primary lesion resulting from NB inhalation of HCl was localized in the most anterior portion of the upper respiratory tract, and no additional injury was detected due to concurrent CO_2 inhalation.

• Inhalation of HCl during MB resulted in high mortality, which was increased with the elevated V_E during concurrent CO_2 inhalation. Lung gravimetric and histologic parameters indicated that the surviving MB animals which were exposed to HCl + CO_2 developed more pronounced lower respiratory tract injury compared to MB animals exposed to HCl alone.

Collectively, the above results suggest that the inhalation of the halides during conditions of increased minute ventilation causes more pronounced injury to the respiratory tract than when the halides are breathed during resting minute ventilation conditions.

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SECTION C

Objective 6: To examine the toxicity of HF when administered directly into the rat's lung.

Results: Demonstration that HF can cause significant injury to the alveolar epithelium and cause pulmonary edema. This finding suggests that the lack of an injurious response to the halides in the alveolar region following their inhalation is due to their efficient removal from the inhaled air stream.

Reports:

Lehnert, B.E., Kinkead, S.A., Kress, J.D., Kober, E.M., G.O. Wood, Stavert, D.M., Brainard, J.R.: Mechanism(s) of lung injury caused by perfluoroisobutylene (PFIB) and related agents. In: Proceedings of the 1991 Medical Defense Biosciences Review. pp. 273-291, 1991.

Brainard, J.R., Kinkead, S.A., Kober, E.M., Stavert, D.M., Lehnert, B.E.: Potential involvement of HF in mechanisms of pulmonary toxicity of perfluoroisobutylene. In: Proceedings of the 1990 Scientific Conference on Chemical Defense Research (in press), 1991.

Brainard, J.R., Kinkead, S.A., Wood, G.O., Stavert, D.M., Lehnert, B.E.: Potential involvement of hydrofluoric acid in perfluoroisobutylene-induced lung injury. 1992 Annual Meeting of the Society of Toxicology, Seattle, WA, February 23-27, 1992.

INTRODUCTION

As previously shown in Section A, the inhalation of the halides at relatively high mass concentrations do not result in major injury in the lung's alveolar region. Various lines of evidence suggest that this may be due to an efficient deposition of the halides from the inhaled air stream prior to the entry of the inhaled air into alveolated structures, i.e., hydrofluoric acid is hydrophilic, it may be efficiently absorbed in the moist upper airways and thereby produce injury in the upper respiratory tract while sparing the more peripheral deep lung. Indeed, based on estimates of airway surface area and measurements of absorption of hydrofluoric acid in the nasal region of the rat, we have recently estimated that essentially all hydrofluoric acid breathed via the oral airway could be removed by the time the inhaled airstream reached 9-10 generations of the rat's tracheobronchial tree (Stavert et al., .991). The possibility, however, has not been ruled out that the alveolar region may be less sensitive to the injurious effects of the halides, which in turn would suggest the existence of a local protective mechanism(s). In conjunction with a study we undertook for the Institute of Chemical Defense, we examined for the toxicity of HF when administered directly into the rat's lung via the intratracheal instillation of different doses of hydrofluoric acid in buffered saline. In addition, we compared the pulmonary injury produced by hydrofluoric acid in acid buffered saline (where the hydrofluoric acid is present primarily as HF) with that in neutral buffered saline (where the hydrofluoric acid is present primarily as F^-) when given by intratracheal instillation.

MATERIALS AND METHODS

Animals: Adult, male Fischer-344 rats weighing between 250 and 280 gm were used in this study. The stock from which the animals were derived was classified as "specific-pathogen-free, virus-free" by the commercial supplier (Harlan Sprague Dawley, Inc., Indianapolis, IN). Upon arrival to our laboratory, the rats were housed two per cage in polycarbonate cages covered with spun polyester filters (DuPont #22 Spinbound Polyester Filter, E.E. DuPont Co., Wilmington, DE) in an animal facility accredited by the American Association for Accreditation of Laboratory Animal Care. The cages were maintained in air conditioned rooms that receive HEPA-filtered air. Water and standard laboratory rat diet (autoclaved) were provided *ad libitum*. Prior to entry into any

experimental study, the rats were maintained for a 2 week period in order to acclimate them to the laboratory facility, as well as to observe them for evidence of disease. In this latter regard, representative animals randomly selected from each shipment group were sacrificed with lethal I.P. injections of pentobarbital sodium (50 mg), blood serum was obtained for antibody titer levels, and their lungs were macroscopically examined for any abnormalities. Animal sera were tested by Microbiological Associates (Bethesda, MD) for Reo 3, GDVII, KRV, H-1, M.AD, LCM, PVM, Sendai, and RCA. All sera tested negative for the above infections. Of relevance, the body weights of the animals used in the experiments were closely matched so their baseline lung gravimetric values would be virtually identical at the time of exposure (Tillery and Lehnert, 1986).

The protocols used in this study were reviewed and approved by the Los Alamos National Laboratory Animal Care and Use Committee. We also note that the conduct and reporting of these studies have been and are in accordance with the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Council (DHEW Publication No. [NIH] 85-23, 1985).

Intratracheal Instillations: The experiments were designed to compare the pulmonary injury produced by four different doses of hydrofluoric acid in buffered saline. In addition, we compared the pulmonary injury produced by hydrofluoric acid in acid buffered saline (where the hydrofluoric acid is present primarily as HF) with that in neutral buffered saline (where the hydrofluoric acid is present primarily as F^-) given by intratracheal instillation. Because the pK_a for hydrofluoric acid is 3.19, we selected conditions of pH=2.1 in phosphate-buffered saline (PBS) to give >90% HF ($[HF]/[F^-] = K_a/[H^+]$), and pH=7.4 to give >99% F^- ($[F^-]/[HF] = [H^+]/K_a$). The hydrofluoric acid in PBS was delivered to the lungs of rats by instillation of 0.5 ml of 0.22 mM, 2.2 mM, 7 mM, 22 mM and 44 mM hydrofluoric acid solutions resulting in dose quantities of 0.11 µM, 1.1 µM, 3.5 µM, 11 µM and 22 µM, respectively. Instillations of PBS, pH=7.4 and PBS, pH=2.1, were also included in the experimental design as controls for the injury produced by instillation of neutral- and acid-buffered saline alone. The intratracheal instillation of PBS solutions was performed with male Fischer-344 rats under Ethrane® anesthesia. Each group of rats consisted of 4-27 animals.

Assessments of Pulmonary Injury: Pulmonary injury was initially assessed by increases in lung wet weight (LWW) and right cranial lobe dry weight (RCLDW) 24 hrs after exposure. The animals were sacrificed 24 hr after the instillations by intraperitoneal injections of pentabarbital sodium and their lungs were excised. The procedures for obtaining lung wet weights (LWW), and right cranial lobe dry weights (RCLDW) have been detailed in Section A. It should be noted that preliminary studies demonstrated that the intratracheal instillation of normal PBS causes no detectable evidence of lung injury, using lung gravimetric measurements and histology as endpoints.

For the electron microscopic analyses of lung injury, groups of animals were anesthetized with pentobarbital sodium (50 mg) 3 hrs after the instillations, their lungs were collapsed by diaphragm puncture, and 4-6 ml of cold fixative (3% glutaraldehyde in 0.1 M phosphate buffer) was instilled via the trachea. The lungs were then excised, submerged in cold fixative, and allowed to fix over night. A 2-3 mm slice through the central portion of the left lung perpendicular to the long axis was removed and further cut into 1-2 mm cubes. These lung fragments were then post-fixed in 1% buffered osmium tetroxide (pH 7.2) and stained *en bloc* with 2% uranyl acetate at 60°C for 15 min. The lung samples were then dehydrated in a graded series of ethanol and propylene oxide, embedded in LX112 resin, and cured over night at 60°C. Tissue blocks were thin sectioned (600-1000 Angstroms thick) and stained with lead citrate. Numerous sample blocks from each rat's lung were sectioned to assure that different regions of the lung were surveyed in our study. Electron micrographs were made using a Philips 410 transmission electron microscope at 100 Kv.

Statistical Analyses: The lung gravimetric data were analyzed for significant differences between groups using a one-tailed t-test (Snedecor and Cochran, 1969).

RESULTS

Lung wet weights and right cranial lobe dry weights determined 24 hours after the instillations are shown in Figures 1 and 2.

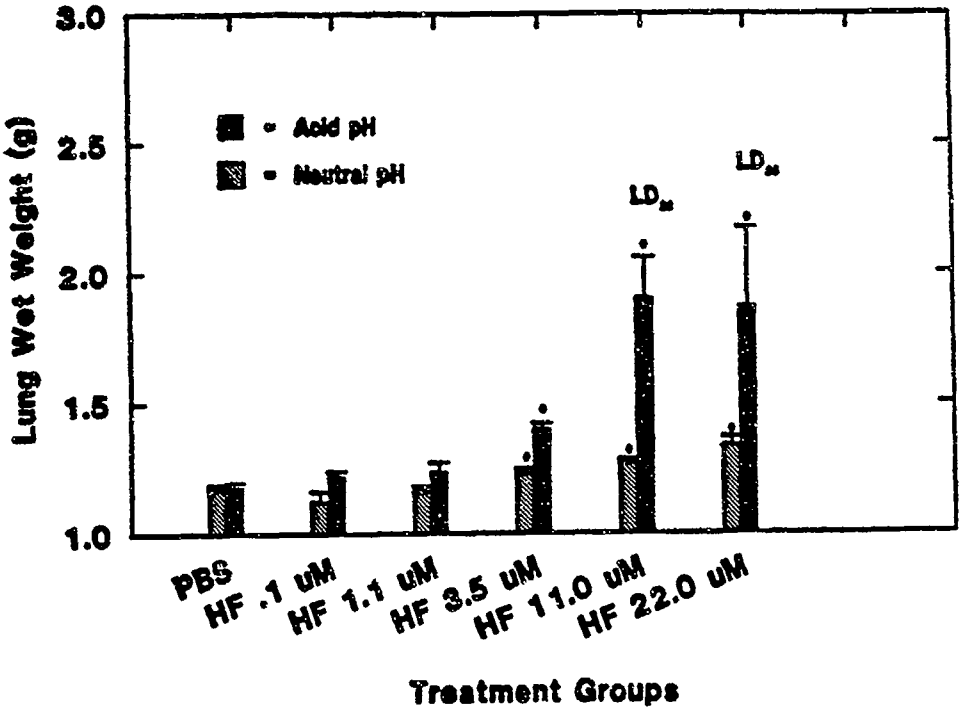

Figure 1: Lung wet weights (LWW) following the instillations of the different HF-containing solutions. *: significantly higher that PBS instilled control lungs, $p<0.05$. Each group consisted of 4-27 rats. The instillation controls consisted of PBS administered at pH values adjusted to 7.4 or 2.1. The LD (lethal dose) values are approximations.

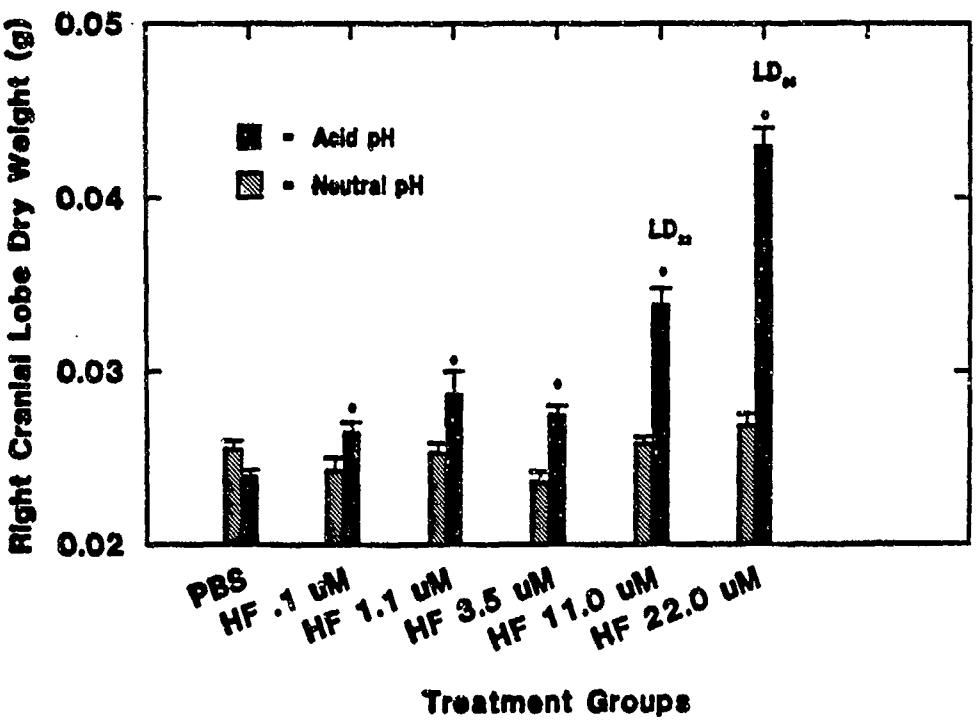

Figure 2: Right cranial lobe dry weights (RCLDW) following the instillations of the different HF-containing solutions. *: significantly higher that PBS instilled control lungs, $p<0.05$. Each group consisted of 4-27 rats. The instillation controls consisted of PBS administered at pH values adjusted to 7.4 or 2.1. The LD (lethal dose) values are approximations.

No significant increases in LWW or RCLDW were observed for rats exposed to either acidic PBS (pH 2.1), neutral PBS (pH 7.4), or for rats exposed to 0.11 μM, and 1.1 μM hydrofluoric acid in either acidic or neutral PBS. Slight but significant increases in LWW occurred after the instillation of 3.5 μM. 11 μM, and 22 μM hydrofluoric acid delivered in neutral PBS (pH 7.4), Figure 1. As previously indicated HF at this pH is >99% F^-. Markedly more pronounced changes in LWW occurred in lungs instilled with HF at a pH of 2.1 when >90% of the instilled sample was undissociated HF. Six out of 27 and 11 out of 13 rats instilled with 11.0 μM and 22 μM doses of HF died within 24 hrs after these instillations, respectively. Accordingly, the lung gravimetric data shown in Figures 1 and 2 were obtained with surviving animals only.

No significant changes in RCLDW were detected following the instillation of HF in neutral PBS, Figure 2. However, significant and generally dose-dependent increases in RCLDW were observed over the dose range of 0.11 μM to 22 μM doses of HF instilled at a pH of 2.1.

Electron Microscopic Observations: No significant evidence of ultrastructural injury in the alveolar region was observed following the intratracheal instillation of PBS at a pH of 7.4 or 2.1 (micrographs not shown). As well, the parenchymal region of the lung did not appear to be significantly affected by the instillation of as much as 22 μM hydrofluoric acid when delivered in PBS at a pH of 7.4, Figure 3A. On the other hand, substantial evidence of lung injury was observed when the hydrofluoric acid was administered in PBS at a pH of 2.1, Figure 3B and 3C. Hallmark features of such injury included the destruction and exfoliation of type I pneumocytes, and the swelling and exfoliation of type II pneumocytes. The appearance of fibrin, amorphous proteinaceous material, and lamellar material in the alveoli were also commonly observed.

DISCUSSION

Because hydrofluoric acid is a weak acid in aqueous solution, the hydrofluoric acid in acid-buffered PBS exists primarily in the undissociated form HF; in neutral-buffered PBS, the hydrofluoric acid exists primarily as F^-. This fact and the lung gravimetric data presented above demonstrate that at 11 and 22 μM doses given by instillation, HF produces more lung injury than F^-. This observation suggests that HF rather than F^- is the agent responsible for injury in pulmonary tissue exposed to hydrofluoric acid. The observations that neither neutral PBS or acidic PBS alone

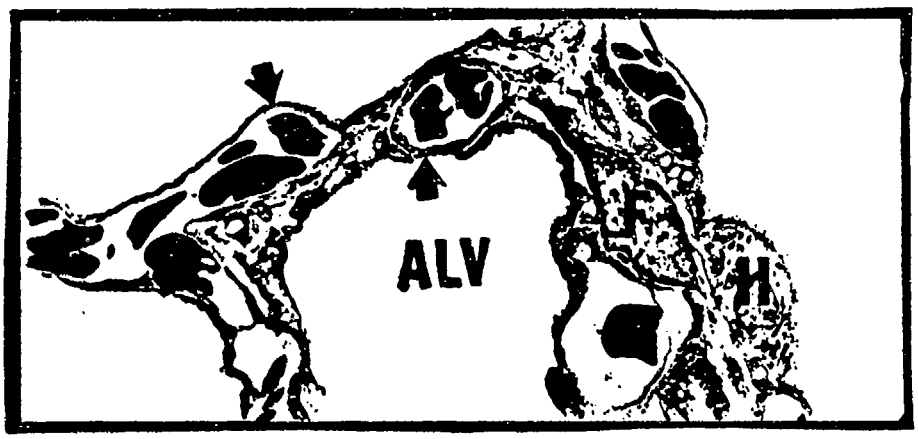

Figure 3A: The alveolar region of a lung that was instilled with 22 μM hydrofluoric acid in PBS, pH 7.4. No ultrastructural evidence of damage to the Type I epithelial cells (arrows) is apparent. The endothelial linings of the pulmonary capillaries are normal in appearance, and no abnormal material, e.g., fibrin, proteinaceous or lamellar material, is present in the alveolar spaces (ALV). A Type II pneumocyte (II) on the alveolar surface and a fibroblast (F) in the alveolar interstitial region show no ultrastructural evidence of injury.

Figure 3B: The alveolar region of a lung that was instilled with 11 μM hydrofluoric acid in PBS, pH 2.1. Type I alveolar epithelial cells show extensive destruction (cytoplasmic rarification and lysis), and many of these epithelial cells or their remnants have lifted off the alveolar surfaces (arrows). An apparently exfoliated Type II pneumocyte (II) is also present in an alveolus. Aside from cutting artifacts, the endothelial cells lining the pulmonary capillaries are normal in appearance.

Figure 3C: The alveolar region of a lung that was instilled with 22 μM hydrofluoric acid in PBS, pH 2.1. Type I epithelial cells show extensive destruction and detachment (small arrows). In some instances, only cell membranes appear to remain. A Type II pneumocyte (II) is swollen and the cytoplasm of this cell shows extensive internal blebbing (open arrow). Cell debris and amorphous proteinaceous material are present in the alveolar spaces (ALV). Endothelial cells are generally normal in appearance.

produced significant lung injury shows that neither the instillation procedure or the delivery of acid (H^+) in PBS results in significant tissue injury. These data argue for the involvement of HF rather than F^- or H^+ as the active species in alveolar injury produced by instilling hydrofluoric acid.

Because hydrofluoric acid is a weak acid in aqueous solution, the hydrofluoric acid in acid-buffered PBS exists primarily in the undissociated form HF; in neutral-buffered PBS, the hydrofluoric acid exists primarily as F^-. This fact and the lung gravimetric data demonstrated that at 11 and 22 μM doses given by instillation, HF produces more lung injury than F^-. That neither neutral PBS or acidic PBS alone produced significant injury showed that neither the instillation procedure or the delivery of acid (H^+) in PBS results in significant tissue damage. Thus, the collective data argue for the involvement of HF rather than F^- or H^+ as the active species in pulmonary injury produced by hydrofluoric acid. Ultrastructural analyses of the lungs instilled with the toxic form of HF revealed marked damage to the alveolar epithelial surface.

The overall results of this study component indicate that HF can be toxic to the alveolar region. Thus, it appears that the lack of an injurious response to the halides in the alveolar region following their inhalation is due to their efficient removal from the inhaled air stream.

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SECTION D

Objective 7: To determine if work performance incapacitation occurs after acute high concentration inhalation of HCl via the nose (NB) or the mouth (MB).

Results: The inhalation of HCl via the nasal or oral routes can result in significant reductions in work performance capacity, as indexed by decreases in maximum oxygen consumption. This effect is more pronounced in nose breathing rats. The reductions in VO_{2max} that occur in mouth breathing rats is biphasic. One mechanism underlying work performance incapacitation following HCl exposure may be increases in airway resistance.

Reports:

Archuleta, D., Schauer, S.M., Stavert, D.M., Lehnert, B.E.: Changes in VO_{2max} and lung injury following exposure of nose- and pseudo-mouth breathing rats to HCl. 1993 Annual Meeting of the Society of Toxicology, New Orleans, March 14-18, 1993.

Objective 8: To assess post-exposure exercise as a potentiator of the severity of expression of halide-induced respiratory tract injury.

Results: No evidence was obtained to suggest that post-exposure exercise can potentite the expression of acute lung injury when HCl is inhaled via the nasal route. However, immediate post-exposure exercise following the inhalation of HCl via the oral route can increase the severity of expression of the injurious response.

Reports:

Archuleta, D., Schauer, S.M., Stavert, D.M., Lehnert, B.E.: Changes in VO_{2max} and lung injury following exposure of nose- and pseudo-mouth breathing rats to HCl. 1993 Annual Meeting of the Society of Toxicology, New Orleans, March 14-18, 1993.

INTRODUCTION

As previously indicated in Section A, we have found that the halides cause injury mainly to the nasal region of the rat when breathed through the nose and mainly injury to the conducting airways of the lower respiratory tract when breathed through the mouth (Kusewitt et al., 1989; Stavert et al., 1991). Edematous processes, the production of airway luminal exudates, as well as the local release of bronchoconstricting mediators in damaged airways would be expected to substantially increase airway resistance and the work of breathing. How such changes may modify work performance capacity is unknown. In this study component, we exposed both nose- and "pseudo-mouth" breathing rats to gaseous HCl as a representative halide and determined how such exposures impact on work performance capacity of the animals, as indexed by reductions in VO_{2max} (Lehnert, 1992). Additionally, we examined whether or not post-exposure exercise can potentiate the severity of expression of halide-induced lung injury.

MATERIALS AND METHODS

Animals: Adult, male Fischer-344 rats weighing between 250 and 280 gm were used in this study. The stock from which the animals were derived was classified as "specific-pathogen-free, virus-free" by the commercial supplier (Harlan Sprague Dawley, Inc., Indianapolis, IN). Upon arrival to our laboratory, the rats were housed two per cage in polycarbonate cages covered with spun polyester filters (DuPont #22 Spinbound Polyester Filter, E.E. DuPont Co., Wilmington, DE) in an animal facility accredited by the American Association for Accreditation of Laboratory Animal Care. The cages were maintained in air conditioned rooms that receive HEPA-filtered air. Water and standard laboratory rat diet (autoclaved) were provided *ad libitum*. Prior to entry into any experimental study, the rats were maintained for a 2 week period in order to acclimate them to the laboratory facility, as well as to observe them for evidence of disease. In this latter regard, representative animals randomly selected from each shipment group were sacrificed with lethal I.P. injections of pentobarbital sodium (50 mg), blood serum was obtained for antibody titer levels, and their lungs were macroscopically examined for any abnormalities. Animal sera were tested by Microbiological Associates (Bethesda, MD) for Reo 3, GDVII, KRV, H-1, M.AD, LCM, PVM, Sendai, and RCA. All sera tested negative for the above infections.

The protocols used in this study were reviewed and approved by the Los Alamos National Laboratory Animal Care and Use Committee. We also note that the conduct and reporting of these studies have been and are in accordance with the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Council (DHEW Publication No. [NIH] 85-23, 1985).

Overview of Experimental Design: Male Fischer-344 rats (SPF, 245-270 g) were lightly anesthetized uniformly with a short-acting anesthetic and either fitted with mouthpieces with attached silastic endotracheal tubes (mouth breathers, MB) or allowed to awaken without further manipulation (nose breathers, NB), as described in Section A and Stavert et al., 1991. After recovery from anesthesia, the NB and MB animals were exposed to 1000 ppm HCl or filtered air only for 30 min. Following the exposures, the mouthpieces and tracheal tubes were removed from the MB animals. Groups of exposed rats were either exercised at varying post-exposure or allowed to rest after exposure until they were sacrificed for lung gravimetric studies using the protocol described in Section A. During the exercise bouts, VO_{2max} was measured as an index of work performance capacity. Data obtained in this study were statistically compared by t test analyses (Snedecor and Cochran, 1969).

Exposure Atmospheres: HCl atmospheres were generated by mixing pure HCl (Matheson Gas, LaPorte, TX) with anhydrous HEPA-filtered air in a stainless steel mixing chamber (Section A; Stavert et al., 1991). The exposure atmospheres were then delivered to the animals using a previously described quartz glass exposure system (Stavert and Lehnert, 1989; 1990). The desired exposure concentration of HCl was determined by quantitatively drawing samples of the atmospheres through midget impingers (SKC Inc., Eighty Four, PA) at a rate of 400 ml/min. Ionic strength adjusting buffer (ISA) or total ionic strength adjusting buffer (TISAB) was used as collection media in the impingers (depending on the electrode used), and samples were analyzed for a given constituent with calibrated, ion-specific electrodes (Orion Research, Inc. Cambridge, MA). A minimum of three samples was collected and measured for every 30 min exposure.

Exercise Protocol: Before use, the rats were subjected to a 20 day training program designed to behaviorally and physically condition them to perform on a treadmill (Stavert and Lehnert, 1989). During the training program, the work intensities and durations of exercise were increased daily until the rats were capable of performing a

"ramp" exercise protocol. Animals that were observed to be "non-runners" during the training sessions were eliminated from the studies. The "ramp" protocol used in these studies began at a treadmill (15% grade) velocity of 10 m · min^{-1}. Every 30 sec, the treadmill velocity was increased by 5 m · min^{-1} up to a final velocity of 60 m · min^{-1}. Maintenance of running speed was encouraged by electro-shock stimulation (40 V, 2 mA) delivered via a grid (Coulburn, Lehigh, PA) mounted behind the treadmill. Prior to the "ramp" runs in the post-exposure experiments, the rats performed a "familiarization run" consisting of two short runs (20 m · min^{-1} for 3 min, 15% grade) separated by a 3 min rest period and finally followed by a 10 min rest period before initiation of the actual "ramp" protocol. Subsequent reference to "exercise" in this report includes the "familiarization run".

Measurement of VO_{2max}: The treadmill used for the "ramp" protocol (Lehnert, 1992; Stavert and Lehnert, 1989) is contained in a metabolic chamber which provides the necessary means to measure O_2 consumption as a rat exercises, Figure 1 (Stavert et al., 1989). Airflow from the chamber (14 L·min^{-1}) was dried (Silica Gel, J.T. Baker Chemical Co., Phillipsburg, NJ) and measured electronically using a pneumotachograph (Fleish No. 0, Gould Inc., Cleveland, OH) and transducer (Validyne Engineering, Northridge, CA) calibrated spirometrically. O_2 content in the effluent airstream is measured with a suitable analyzer (Ametek S-3A O_2 Analyzer, Ametek, Pittsburg, PA) that is calibrated with a primary gas standard (National Bureau of Standards grade, Matheson Gas, LaPorte, TX). O_2 consumption is calculated every 5 sec via a data acquisition and computer system (HP-3497A, Hewlett-Packard, Corvallis, OR) using the equations of Mautz et al. (1985). VO_{2max}, or the plateau of O_2 consumption achieved with increasing work loads, is expressed as ml O_2/kg body weight/min following correction for standard temperature and pressure. Trained rats usually maximally consume O_2 ~5-6 min after the "ramp" protocol is initiated (Stavert and Lehnert, 1989).

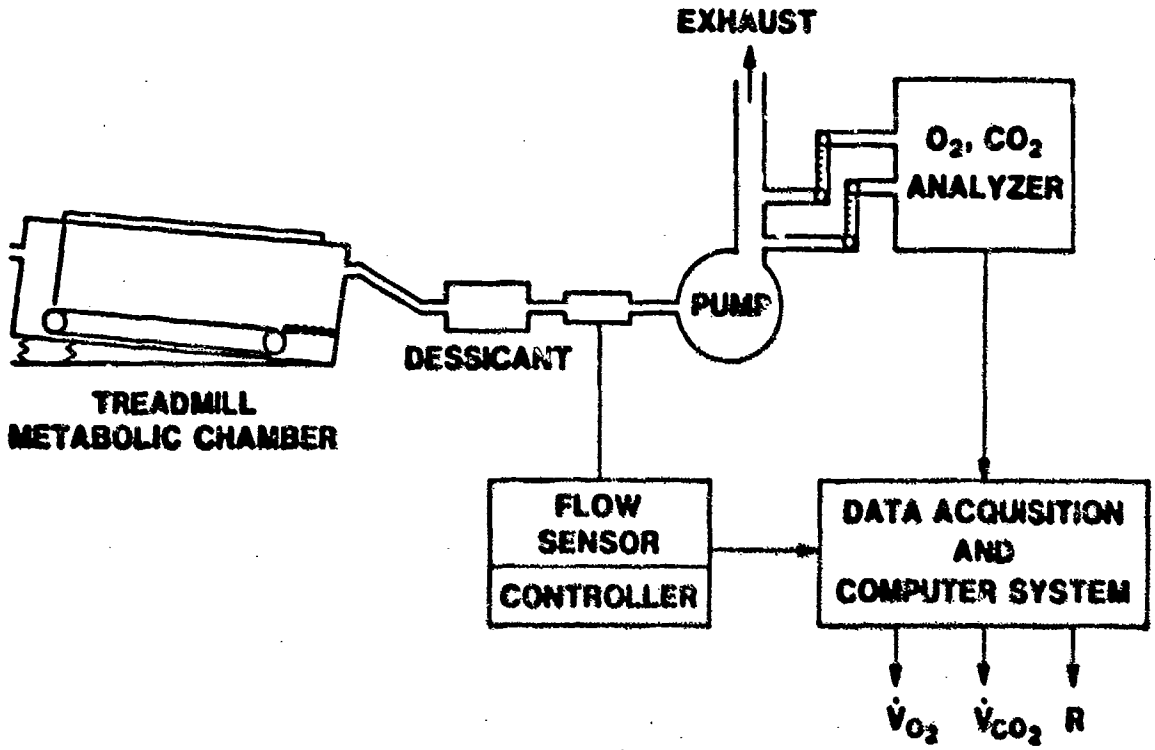


Figure 1: Schematic representation of the treadmill-metabolic system used for measuring VO_{2max}.

RESULTS

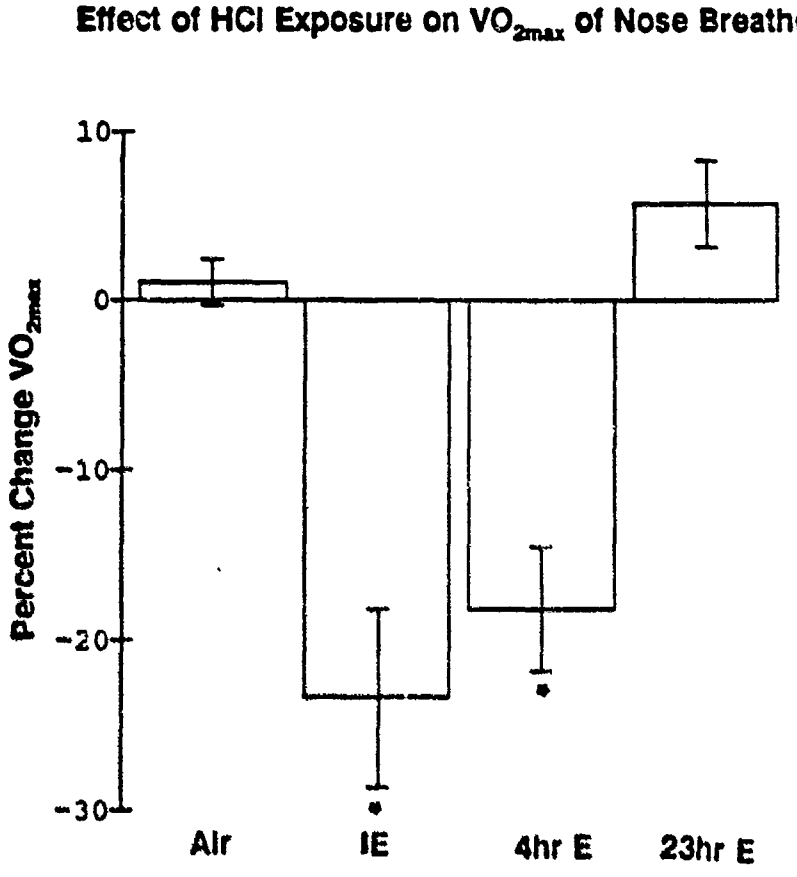
Post-Exposure Changes in VO_{2max} Following HCl Exposure: When normal, obligatory nose-breathing (NB) rats were subjected to exercise immediately after exposure to the HCl, their maximum oxygen consumption was reduced by ~23% from air control values, Figure 2. Though somewhat less diminished, VO_{2max} values of the HCl exposed, NB rats remained reduced when rats in a second group were exercised 4 hrs after exposure. However, when another group of HCl exposed, NB rats was exercised 23 hrs after exposure, their VO_{2max} values were not significantly different from those obtained from air exposed control rats, Figure 2.

A more complicated, biphasic pattern of reductions in VO_{2max} was obtained with the pseudo-mouth-breathing (MB) rats after exposure to the HCl, Figure 3. Similar to the NB animals, VO_{2max} was reduced by ~18% when the MB animals were exercised immediately after exposure. However, when another group of the MB animals were subjected to the ramp protocol as of 4 hrs after HCl exposure, no evidence was obtained that indicated a compromise in work performance capacity at this post-exposure time. Yet, the VO_{2max} values of the MB rats were again observed to be reduced from air control values (~12%) when maximum oxygen consumption was measured as of 23 hrs with a third group after exposure, Figure 3.

Post-Exposure Exercise Potentiation of Lung Injury: For this part of the study, the animals that were exercised immediately after exposure to the HCl were sacrificed as of 1 hr post-exposure for lung gravimetric measurements, and the groups of rats that were exercised as of 4 and 23 hrs after exposure for the VO_{2max} measurements were sacrificed for the lung gravimetric measurements as of 24 hrs after the HCl exposures. Control groups of rats consisted of air exposed rats and NB and MB rats that were allowed to rest after the HCl exposures prior to being sacrificed for the lung gravimetric studies. The parameter used to index pulmonary edema in this study was the lung wet weight to body weight ratio. We selected this manner of data presentation in that the body weights of some of the shipment groups of rats used in the study were unusually non-uniform. Thus, we could not assume that the pre-exposure lung weights of the rats across all the groups were statistically similar (Tillery and Lehnert, 1986).

As summarized in Figure 4, exercise performed by NB rats, whether performed immediately after HCl exposure or at later post-exposure times, did not result in changes in lung wet weight/body weight changes beyond those observed with HCl exposed but rested

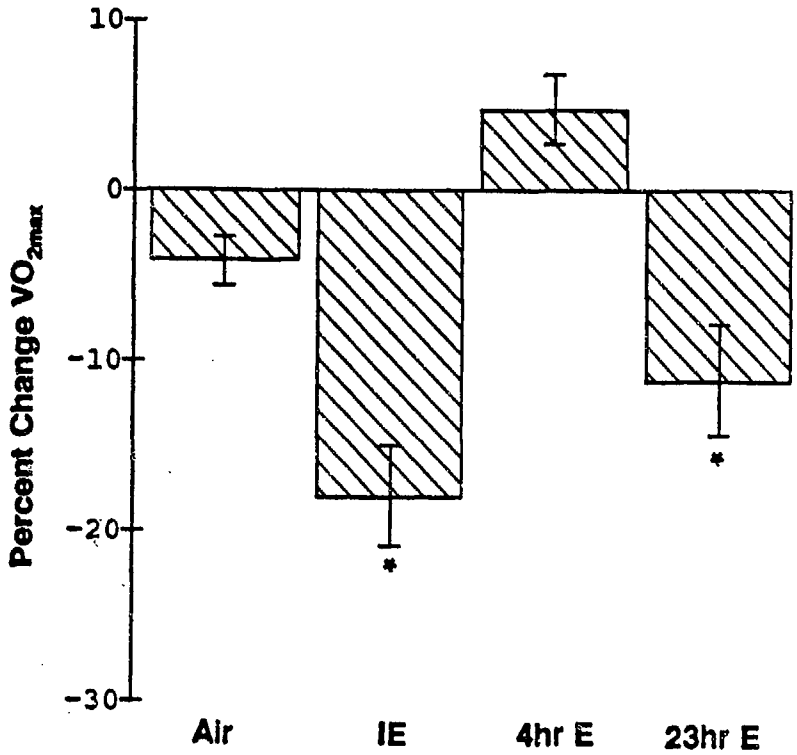
Figure 2



Percent change in maximum oxygen consumption (VO_{2max}) at various times following exposure to 1000 ppm HCl for 30 minutes. Values represent the mean and standard error of the mean of n=3 to 9 animals. (*) indicate significant difference compared to the air control, $p \leq 0.05$.

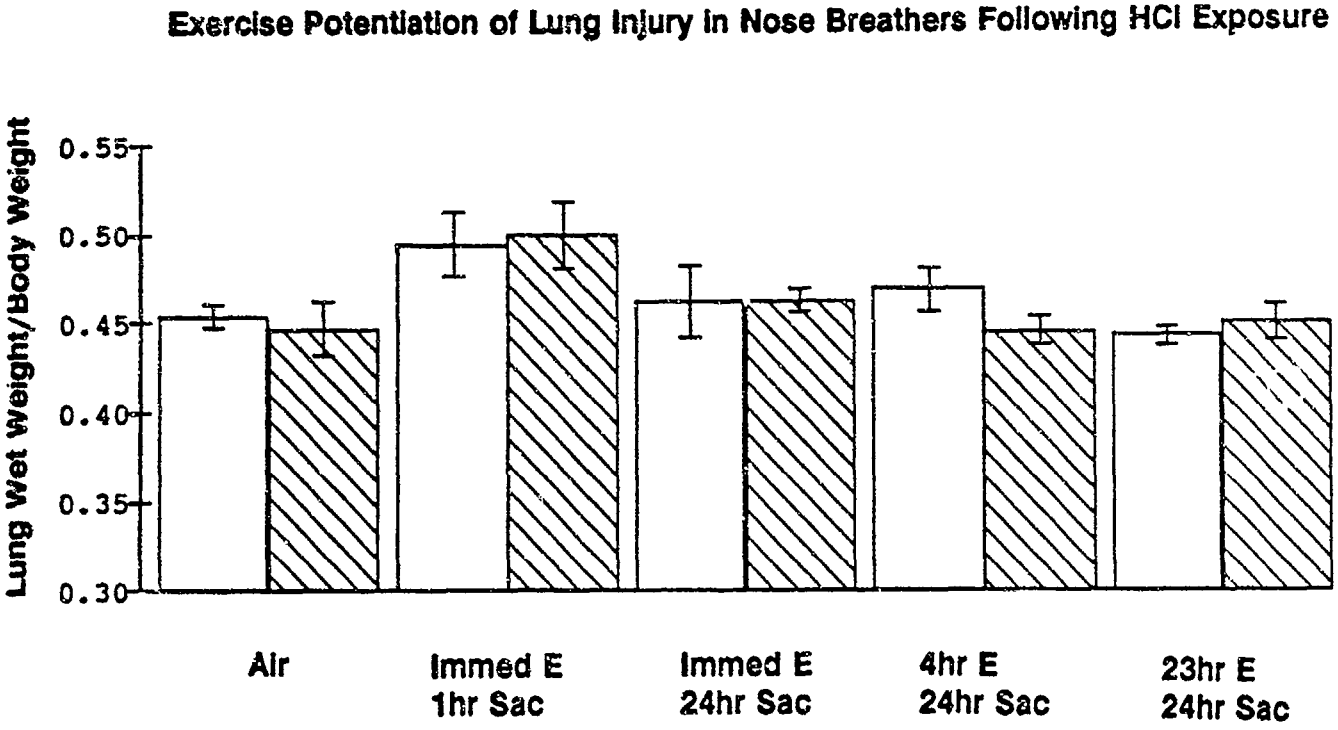
Figure 3

Effect of HCl Exposure on VO_{2max} of Mouth Breathers



Percent change in maximum oxygen consumption (VO_{2max}) at various times following exposure to 1000 ppm HCl for 30 minutes. Values represent the mean and standard error of the mean of n=5 to 27 animals. (*) indicate significant difference compared to the air control, $p \leq 0.05$.

Figure 4



Lung wet weight (LWW) as a function of body weight in rested and exercised animals at various sacrifice times following exercise at various times after exposure to 1000 ppm HCl for 30 minutes. Values represent the mean and standard error of the mean of n= 3 to 11 animals. There is no significant difference between rested and exercised groups.

rats. [Note: this parameter would be expected to increase with increasing severity of pulmonary edema.] With the MB rats, however, some evidence that post-exposure exercise can potentiate the injurious response was obtained with the animal group that was sacrificed shortly after immediate post-exposure exercise, Figure 5.

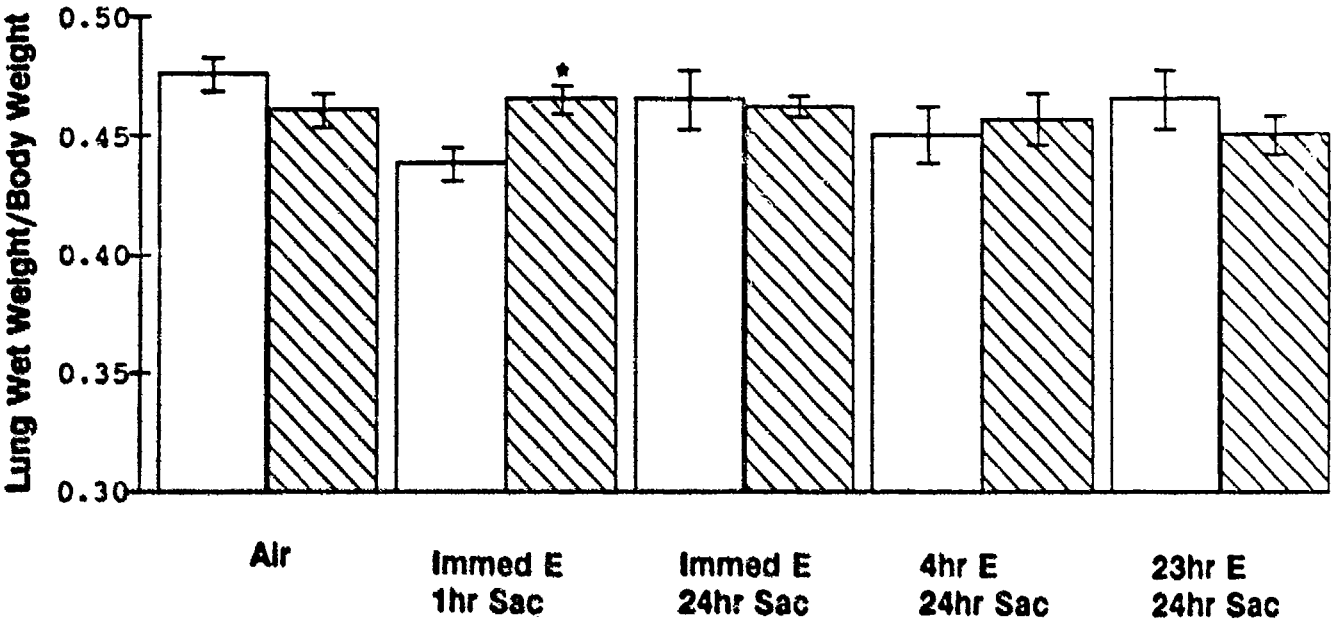
DISCUSSION

The results from this study demonstrate that the inhalation of HCl as a representative halide can result in post-exposure decreases in work performance capacity, whether the HCl is breathed through the nose or via the oral pathway, with the most pronounced decreases in VO_{2max} occurring immediately after the exposure. This response appeared to be most marked in NB rats. In that HCl is primarily an upper airway irritant (Morris and Smith, 1982) and it causes injury primarily to the nasopharyngeal region in nose-breathing rats and injury to the conducting airways in pseudo-mouth breathing rats (Kusewitt et al., 1989; Stavert et al., 1991), it is tempting to speculate that the reductions in work performance capacity observed in this study was due mainly to increases in airway resistance. However, it is of interest to note that with the MB rats, the post-exposure reductions in work performance capacity were biphasic. While the underlying explanation for this response pattern requires further investigation, it does suggest the possibility that reductions in work performance capacity following the inhalation of HCl via the oral route may have more than one mechanistic cause. This is not an unreasonable expectation, given the numerous physiological factors that contribute to maximum oxygen consumption.

Lastly, no evidence was obtained to suggest that post-exposure exercise can potentiate the severity of lung injury when HCl is inhaled through the nose. This finding is consistent with our previous findings that the injurious response to HCl in nose-breathing rats is confined to the nasopharyngeal region of the respiratory tract (Stavert et al., 1991). We did, however, obtain some evidence that the severity of the injurious response to HCl inhaled via the oral pathway can be potentiated by post-exposure exercise. Using the lung model of Yeh et al. (1979), we previously estimated that halides that are inhaled by mouth breathing rats can reach airway generations 9 or 10 (Stavert et al., 1991), where injury would be expected. Thus, it is possible that post-exposure exercise results in the potentiation of the edematous response to HCl in the conducting airways. Unfortunately, little information is available to date regarding all of the mechanisms by which post-exposure exercise can exert a detrimental effect on toxic gas-induced injury.

Figure 5

Exercise Potentiation of Lung Injury in Mouth Breathers Following HCl Exposure



Lung wet weight (LWW) as a function of body weight in rested and exercised animals at various sacrifice times following exercise at various times after exposure to 1000 ppm HCl for 30 minutes. Values represent the mean and standard error of the mean of n = 3 to 22 animals. (*) indicate significant difference between rested and exercised groups, $p \leq 0.05$.

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SECTION E

Objective 9: To initiate studies to determine whether or not lung injury becomes more pronounced after halides are breathed in combination with a particulate phase.

Results: No evidence was found to indicate that the inhalation of a halide (HCl) along with particulate carbon results in significant alveolar damage as assessed by lung gravimetric and histopathologic criteria.

Reports:

Oberdörster, G., Ferin, J., Finkelstein, J., Baggs, R., Stavert, D.M., Lehnert, B.E.: Health hazards from thermal degradation events: Particulate vs. gas phase effects. Intern. Conference on Environmental Systems, Aerospace Medical Association, Seattle, WA, July, 1992.

INTRODUCTION

Numerous studies have demonstrate that the injurious effects of a variety of toxic gases are more pronounced when exposures occur concurrently with a particulate phase component (e.g., Boren, 1964; Kulle et al., 1986; Last et al., 1983). The mechanistic bases for such observations are potentially numerous. Under some conditions, at least, particles serve as carrier vehicles, which can enhance the deposition of adsorbed gases at sites in the respiratory tract where the particles preferentially deposit. In other instances, particulate materials that are generally considered to be relatively benign or noncytotoxic can cause an injurious response in addition to that caused by the inhaled gas when the particles fall within the ultra-fine range.

We undertook the development of aerosol technology in our laboratory (in collaboration with the University of Rochester) so that issues concerning the toxicity of atmospheres containing toxic gases and particles can be investigated. As indicated in the FY 92 Statement of Work, the initial specific objective of this effort was to determine how the profile and severity of injury to the respiratory tract following halide exposure may be altered by the concurrent inhalation of aerosolized particles. For this study component, we used gaseous hydrogen chloride as the test halide inasmuch as HCl, HF, and HBr all appear to manifest comparable toxicities in the respiratory tract (Stavert et al., 1991).

MATERIALS AND METHODS

Exposures: This preliminary study was mainly undertaken to establish methods to generate and deliver exposure atmospheres consisting of halide gas and aerosolized particles to rats. Three exposure groups of Fischer-344 rats (SPF) were used in this study, N=6 per group. Exposures were conducted while the animals (normal nose breathers) were positioned in whole body exposure tubes. Twenty min exposures were conducted with exposure atmospheres consisting of either 1000 ppm HCl, carbon particles (~0.8 μm geo. diam.), or 1000 ppm HCl + carbon particles. After cessation of the exposures, the animals were returned to their cages for a 24 hr period prior to sacrifice by lethal injection with pentabarbital sodium. HCl only exposure atmospheres were generated by mixing pure HCl, (Matheson Gas, LaPorte, TX) with anhydrous HEPA filtered air in a quartz-glass mixing chamber and inhalation chamber. Exposure concentrations of HCl were determined by quantitatively drawing samples of the atmospheres through midget impingers. Ionic strength adjusting buffer, (ISA) was used as collection medium in the

impingers, and it was analyzed with with calibrated, ion specific electrode, (Orion Research Inc. Cambridge, MA), as described in Section A. A minimum of 3 discreet samples was measured for every 20 min exposure. The mean HCl concentration for the HCl only exposures was 1016 ± 21 ppm. Particulate carbon only atmospheres were generated via a aerosol generator (Jet-O-Miser Model 00, Fluid Energy Processing and Equipment Co., Hatfield, PA), which was fed carbon fine particles from a powder feeder (Accurate Model 102 Feeder, Accurate, Whitewater, WI). Exposure atmosphere particulate mass concentration was determined by filter analyses. Exposure atmospheres were combined using the same methodology described above for the rat group that received the carbon particles + HCl atmosphere. The mass concentration of carbon was ~178 mg/M^3, but some variation occurred during the actual exposures.

Endpoints of Toxicity: Endpoints examined in this study were lung gravimetric changes and histopathologic changes in the lungs.

RESULTS

The lung wet weights (LWW) after exposure to HCl only, particulate carbon only, and HCl + particulate carbon were 1.179 ± 0.031 g, 1.20 g ± 0.027 g, and 1.20 ± 0.023 g, respectively. Non of these values were significantly different from one another. Right cranial lobe dry weights (RCLDW) for these groups were also closely similar, 0.023 ± 0.0007 g, 0.024 ± 0.0004 g, and 0.020 ± 0.0013 g, respectively. Light microscopic assessments of the lungs from the animals in each group revealed no significant differences.

DISCUSSION

The lack of a demonstrable effect due to the co-inhalation of particles and HCl may have been due to many variables. 1) Some variability was experienced in maintaining a stable mass concentration of the particles during the exposures. 2) The mass concentration of particles, which was below that expected to occur in a real fire scenario, may have been too low. 3) The aerodynamic size distribution of the particles may not have been optimal to favor alveolar deposition. 4) The breathing patterns of the animals may have differed with the various exposure atmospheres. On the other hand, it remains possible that carbon particles do not serve as carrier vehicles for halides to more peripheral lung regions or that any HCl that may have adsorbed onto the particles desorbed prior to particle deposition into

the alveoli. Regardless, this study led to the establishment of exposure protocols for the co-administration of test atmospheres that contain both gaseous and particulate phases.

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